Seed Handling Guidebook

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Proper seed handling techniques are necessary for successful reforestation efforts. With the short supply of seeds from some sources, higher cost of seed orchard seeds, and the legislated use of seeds of the best genetic quality available in BC, there is good reason to be as efficient as possible with tree seeds. This guidebook is intended for use by individuals who handle cones or seeds (collectors, orchardists, processors, and nurseries) and by those who would benefit from an integrated view of seed handling—from cone collection to sowing seeds in nurseries (seed owners, reforestation foresters, certification agencies). The guidebook focuses on north temperate conifers, particularly from the Pacific Northwest, but the general principles are applicable to most conifer species. Discussion of procedures are based on those at the BC Ministry of Forests Tree Seed Centre and supporting facilities or functions. Exact procedures may differ by facility, but this guidebook will be focused more on principles than exact procedures. Most of us deal with only part of this seed handling system, but it is important to understand the full spectrum of seed handling activities. Poor handling by others at any previous or subsequent stage may negatively impact your product!

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This guidebook is intended to provide readers with information on seed handling with an emphasis on three topics:

- **Guidelines for proper seed handling**
- **The tools required to recognize seed problems**
- **Techniques for avoiding or correcting seed problems.**

The guidebook provides some background information on seed condition, seed insects, and seed fungi and then covers the 'seed handling system' that encompasses all seed handling activities from cone collection to sowing in the nursery (Figure 1). The system begins with the collection of cones from seed orchards, wild stands, or plantations. This guidebook does not discuss cone crop development or monitoring. For information on these topics readers are referred to *Forest Tree Seed Production* (Owens and Blake 1985), *A Guide to Collecting Cones of British Columbia Conifers* (Eremko et al. 1989); *A Field Guide to Collecting Cones of British Columbia Conifers* (Portlock 1996), or Chapter 15 in *Regenerating British Columbia's Forests* (Leadem et al. 1990).

Post-collection handling, including temporary storage, monitoring and transport of cones to a processing facility, is a key step in the production of high quality seeds. Unfortunately it is a stage that too often receives inadequate attention. It is generally recommended that the cones of most species should be field-stored (interim storage) for approximately four weeks prior to shipping to the extractery to reduce moisture content and risk of damage. However, exceptions to this rule include western hemlock[1] and western redcedar which have shallow seed dormancy and should be shipped to the seed processing facility immediately after picking. Cone storage or conditioning may continue at the cone and seed processing facility if moisture content is still too high or if cones are not scheduled for immediate processing.

Cones are generally opened through a kilning process and the seeds extracted by tumbling or screening. Cones of *Abies* spp. may not be kilned at some facilities as their cones naturally disintegrate with additional conditioning. The drying process and separation of seeds from cones, by either method, constitutes cone processing.

Seed processing involves the removal of debris, removal of non-viable seeds, and reduction of seed moisture content that prepares the seeds for long-term storage. Many different processes and pieces of equipment may be used in seed processing. For example, all Pinaceae species have their seed wing removed during processing. However, the seed wing is not removed in the Cupressaceae species since it would significantly damage the seeds.

Seed testing is an important step as it quantifies seedlot quality and allows one to estimate the number of seedlings that may be obtained (potential seedlings) from a quantity of seed. Legislated requirements to ensure seed quality for testing are in place and acceptable values for purity and moisture content must be met before registration can occur. Testing provides the basic seedlot information that will help manage the use of a seedlot.

[1] Scientific names, common names, and abbreviations of BC conifer species are presented in Appendix 1.

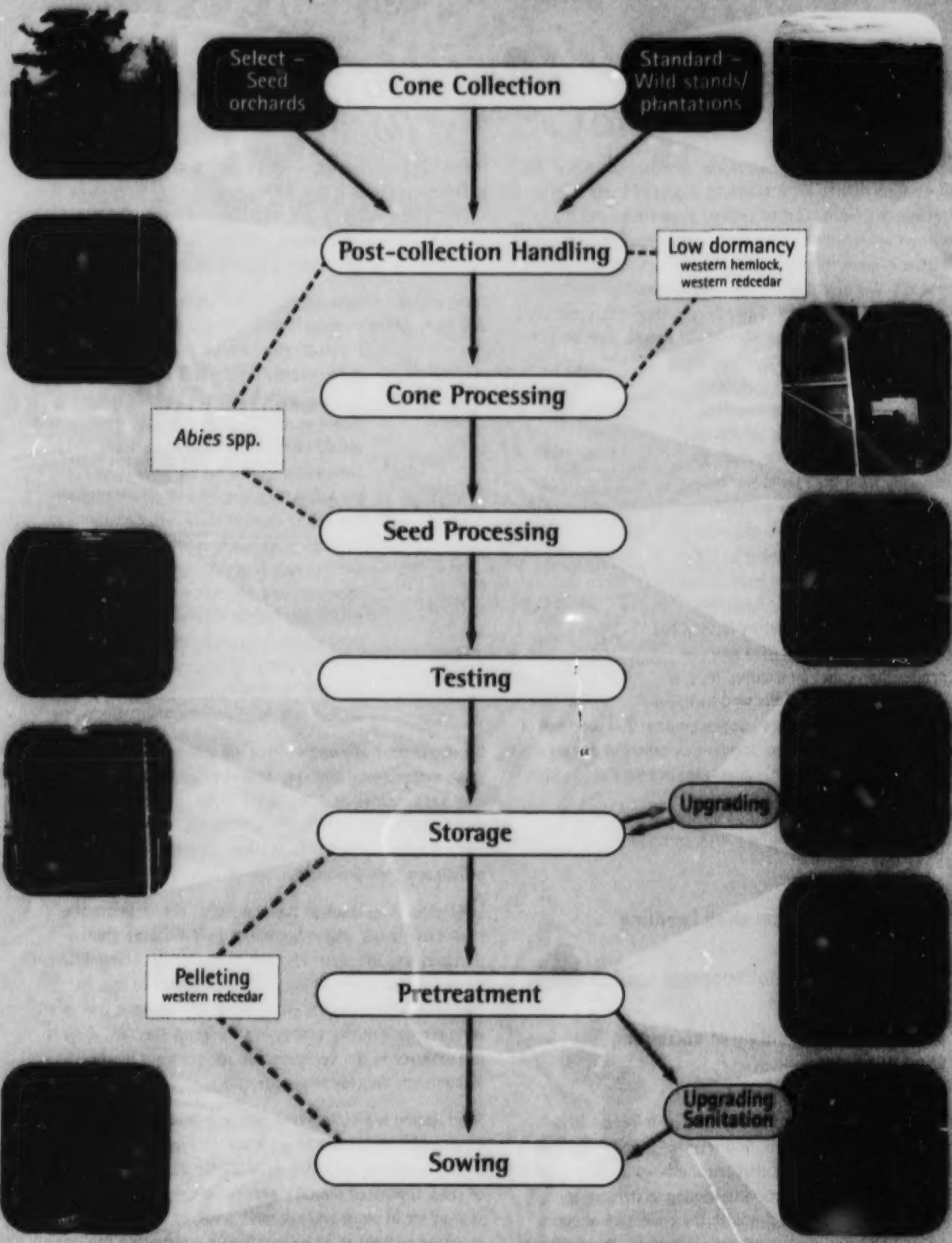
Select – Seed orchards
Cone Collection
Standard – Wild stands/ plantations
Post-collection Handling
Low dormancy
western hemlock, western redcedar
Cone Processing
Abies spp.
Seed Processing
Testing
Upgrading
Storage
Pelleting
western redcedar
Pretreatment
Upgrading Sanitation
Sowing

Figure 1

Seeds remain in long-term storage (-18°C) until they are required for sowing. Fortunately, conifers exhibit good storability under appropriate conditions. During storage, seedlots will be retested for germination and may be upgraded for purity, moisture content, or germination and placed back into long-term storage.

Seeds are withdrawn for reforestation and pretreated with the appropriate techniques to maximize germination capacity and rate. All species, except western redcedar, are initially soaked in running water and given a period of cold stratification to remove dormancy, increase the speed and uniformity of germination, and improve their ability to germinate under sub-optimal conditions. Following partial or full stratification, it may be possible to upgrade seedlots through various procedures to eliminate poor seeds and thereby increase the overall quality of seeds sown in the nursery.

Western redcedar seeds are not dormant and do not require cold stratification. Because of their lightweight, irregularly shaped seeds and wings, which are not removed during seed processing, western redcedar seeds are pellet-coated to facilitate mechanical sowing in the nursery. Nursery sowing is generally performed mechanically and one or more seeds may be sown per cavity depending on seed quality and nursery policy. Seed sanitation procedures (e.g., hydrogen peroxide soaks) may also be implemented before, during, or after soaking, or at some point during stratification.

Chapters entitled "Germination Environment" and "Nursery Germination" are also included in this guidebook to complete the seed story and extend available information on these important topics. These sections are specific to the container system used throughout BC and most of Canada. The sowing aspect of the seed handling system is quite different in bareroot seedling production, which has been virtually eliminated as a method of growing seedlings for reforestation in BC.

The seed handling system illustrates the many facets of handling—showing how handling at each stage affects the next stage and ultimately the quality of the final seedlot. A key element to improving knowledge of seed handling is the sharing of hands-on experience and research on conifer tree seeds. While seed value has increased dramatically in most jurisdictions, the amount of seed research being performed and the pool of experienced seed technicians has decreased in North America (Smith 1998; Bonner 1996). By understanding the entire seed handling system, it is believed that efficiencies can be gained in seed use.

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The information that follows is presented in a practical fashion with the intention of bringing all readers to a common level of knowledge. Technical terms will be indicated in **bold** print for their first usage and are defined in the glossary (Appendix 2).

Seed condition refers to the physical and physiological state of being and health of seeds. Seeds are a living biological end-product of genetic and environmental interaction and their behaviour cannot be predicted with certainty (Ben Wang, pers. comm., May, 2000). Seed condition explains the large variability we observe between species and seedlots, and even within seedlots.

Seed condition may be characterized qualitatively through visual observation (e.g., cutting tests or x-rays) or quantitatively through seed testing (e.g., moisture or germination tests). Within the seed handling system, seeds are removed from cones, dewinged, separated from foreign debris and culled if non-viable, prior to being termed a seedlot. As this processing advances, the seeds begin to appear more streamlined, cleaner and no longer mixed with extraneous material. Seeds ready for storage carry little if any debris with them.

Seeds are a living biological end-product of genetic and environmental interaction and their behaviour cannot be predicted with certainty

Seeds—even clean seeds—can be passive carriers of pathogens that can kill or mummify them. Even after planting, these pathogens can emerge under suitable environmental conditions and be further transmitted. Fungi, insects, bacteria, viruses, and nematodes are associated with and can be carried with, on, and in all seeds. Of these organisms, insects and fungi are most important for conifer seeds. They are discussed in the chapters "Cone and Seed Insects" and "Seed Fungi."

Visual Observation

The visual observation of seeds is an important step in assessing condition and determining if a problem exists. The technique requires no special tools and provides an instantaneous assessment of seed condition. While evaluation based on observation will always be subjective, it is through experience gained from repeated observation, assessment, and follow-up, that the full benefits of observations can be realized. Discussion of observation techniques will cover basic external and internal seed characteristics. A more complete coverage of seed characteristics is available in *Anatomy and Morphology of Conifer Tree Seed* (Kolotelo 1997).

Morphology

The exterior of a seed is comprised of the seed coat, which is composed primarily of dead cells and acts as a protective covering to the inner living tissues. The appearance of the seed coat can be quite variable (Figure 2). Lodgepole pine and interior spruce seeds are similar in size, are both completely dewinged during processing and do not possess resin vesicles.

Figure 2 A comparison of the exterior appearance of interior spruce (Sx), interior lodgepole pine (Pli), Douglas-fir (Fd), and western redcedar (Cw) seeds.

These species can usually be differentiated based on seed size and seed coat colouration. Douglas-fir seeds do not possess resin vesicles, but the seed wings are not completely removed due to their integral connection with the seed coat—a small remnant of the wing can usually be seen following processing. Western redcedar seeds are quite distinct as the seed wings are not removed and resin vesicles are present on the seed surface.

Resin vesicles are present in all species of *Abies*, *Thuja*, and *Tsuga*. Although their role is unclear, studies have confirmed that damage to resin vesicles will result in decreased germination (Kitzmiller et al. 1973; Gunia and Simak 1970). Removal of the outer seed coat layer during handling can greatly change the appearance of seeds and predisposes the vesicles to damage (Figure 3). Seeds that have had their resin vesicles damaged can be identified by one or more of the following characteristics: a distinct odour, a tacky or pitchy feel to the seed, a greyish coloration, or a smoother, duller appearance to the seed (Figure 4). Damage to vesicles will occur as a result of rough handling of cones and seeds at any stage in the seed handling system.

...damage to resin vesicles will result in decreased germination

Figure 3 The appearance of Amabilis fir (Ba) and western hemlock (Hw) seeds that are a) intact or b) with the outer seed coat layer removed exposing the resin vesicles to potential damage.

Figure 4 Resin vesicle damage in Amabilis fir (Ba).

The moisture status of the seed coat is an important visual attribute used to gauge surface drying of seeds. Once the inner components of the seed have been fully hydrated in preparation for stratification or sowing, surface drying is generally used to remove excess moisture. This drying minimizes potential fungal buildup, allows increased oxygen levels to reach the embryo and ensures that the seed is easy to sow mechanically. When excessively moist, seeds of many species appear dark compared to seeds which have had their surface moisture removed (Figure 5).

Figure 5 Interior spruce seeds showing imbibed seeds with (darker) and without (lighter) surface moisture.

Anatomy

Observations of the seed coat can provide some insight into seed condition, but an assessment of the internal components is required for a complete picture of seed condition. The internal components of seeds can be viewed by cutting a seed and examining it under magnification. These seed anatomy or cutting tests provide basic information on the quality of seeds at different times in the seed handling system. During collection, cutting tests indicate the proportion of viable seeds and their degree of maturity. During cone processing, cutting tests are used to assure all viable seeds are removed from cones. During seed processing, cutting tests are used to fine tune equipment settings and ensure viable seeds are being retained and non-viable seeds are being removed from a seedlot. Cutting tests can also be used to assess a seedlot following a germination test if it is below the species average in quality. To obtain the best results from the cutting test, ensure that the seed is imbibed first (fully hydrated) in a water bath (see "Seed Testing" chapter, Table 5, page 46, for soaking times). As the components swell, tissues become more differentiated making it easier to identify viable seeds in the hydrated condition. Cut the seed on its longest axis through its thinnest dimension (Figure 6). Seed anatomy can be best

...an assessment of the internal components of seeds is required for a complete picture of seed condition

Figure 6 When performing cutting tests, cut the seed longitudinally through its thinnest dimension.

viewed when the cut has been made exactly through the middle of the side. The anatomical detail is reduced as the cut moves off-centre. For some categories (i.e., rotten contents) it is not as important to perform a 'perfect' cut, but slight differences in anatomy (i.e., obtaining an accurate estimate of embryo length) require a centred cut. Cutting tests generally become more challenging as seed size decreases (i.e., more difficult to get good sections in western hemlock as compared with ponderosa pine). While a hand lens is sufficient, a dissecting microscope will greatly increase your power of observation.

The cut seed is classified into categories based on the observed anatomy. A random and representative seed sample is key to properly assessing a seedlot. An assessment of the number or proportion of seeds in each category will allow the seedlot to be characterized to estimate germination capacity and provide guidance about what procedures can be used to improve seed quality throughout the seed handling system. The categories used will depend on the stage in the seed handling system, level of detail required, and types of seeds observed. For example, a cutting test on seeds just extracted from the cones should include a group for empty seeds, but this category is usually absent from fully processed seeds of most species. The number of seeds to use is also debatable and depends on the level of detail required, but sample sizes of between 25 and 50 seeds are most commonly used.

The three main components of the seed, **seed coat**, **megagametophyte**, and **embryo**, can all be viewed within the cut seed (Figure 7). The seed coat in conifers is generally found to have three distinct layers and is mainly composed of dead cells. The seed coat protects and insulates the inner tissues from damage. The megagametophyte tissue serves as the food reserves for the embryo and may also be implicated in seed dormancy. The major portion of the megagametophyte is composed of lipids, which are water insoluble and efficient at storing energy for the demands of germination. The third component is the embryo which will give rise to the future seedling. Structures within the embryo include the **cotyledons** (sometimes called seed leaves) which will function in harnessing energy for growth through photosynthesis immediately after germination; the **shoot apical mersitem** which through cell division will give rise to all subsequent vegetative tissues; the **root apical meristem** which will give rise to all below-ground structures; a **rootcap** to protect the root apical meristem, and the **procambium** which will give rise to the vascular tissues important in translocating water, sugar and minerals within the plant. The **pith** is found inside, and the **cortex** on the exterior, of the procambium, but both tissues are composed of **parenchyma** cells.

It is usually relatively easy to identify seeds that can be considered potentially viable, but classifying or determining the cause of a problem can be more challenging

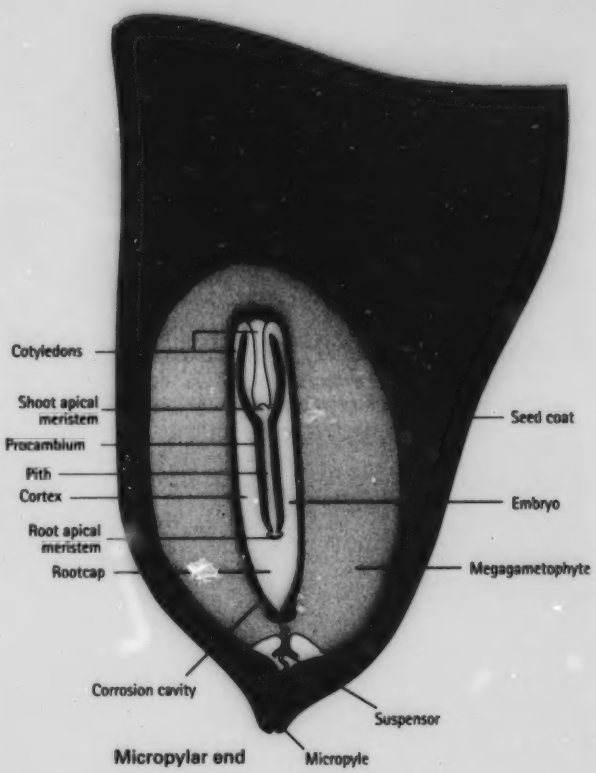

Figure 7 The anatomical details of a generalized conifer seed in longitudinal section.

It is usually relatively easy to identify seeds that can be considered potentially viable, but classifying or determining the cause of an aberrant condition can be more challenging. Representative cut seeds of the four major reforestation species in BC are illustrated in Figure 8.

Figure 8 A comparison of longitudinal sections of interior spruce (Sx), interior lodgepole pine (Pli), Douglas-fir (Fd), and western redcedar (Cw) seeds.

In Figure 9, a key is presented showing one method for classifying seeds. Seeds described as having rotten contents generally have a loose, unorganized and discoloured appearance (embryo may or may not be identifiable). These seeds are easy to identify and, due to an advanced state of deterioration, will not germinate. Some activities that can increase deterioration are physical damage (bruising); overheating (post-collection storage or in transport); prolonged storage of moist seeds (>10% moisture content); and the presence of micro-organisms. The most commonly observed categories are described, but it should be appreciated that seed condition is continuous and these categories are created for our convenience.

Seeds with developmental problems can be defined, for simplicity, as seeds with immature components. An immature embryo is the most readily identified component as it occupies only a fraction of the corrosion cavity which forms within the megagametophye. A cone crop is generally considered morphologically mature when its embryo occupies more than 90% of the corrosion cavity. Immature seeds may contain a normal looking megagametophyte and may germinate if they have matured sufficiently. While the threshold between germination and continued deterioration varies, it probably lies between 60 and 75% of the length of the corrosion cavity. Seeds with embryos between 75 and 90% have a high probability of germinating, but germination rate, vigour, and storability may be reduced compared to mature seeds. Immature seeds that contain a deteriorated megagametophyte probably will not germinate.

Potentially viable seeds possess all essential structures including a mature embryo and a uniform, firm, white megagametophyte without any signs of deterioration (Figure 7). These seeds will germinate promptly, with appropriate dormancy breaking treatments, and will likely store quite well. Seeds with a slightly deteriorated megagametophyte will probably germinate if provided with 'good' germination conditions. The practical impact of deterioration can be profound if germination is slowed, through unsuitable germination conditions, to the point where insufficient storage reserves are available to fuel germination. As deterioration of the megagametophyte worsens, the probability of germination decreases.

This is but one example for classifying seeds and many other methods may work well for your situation. An example of a cutting test scoresheet is presented in Figure 10 (page 9). The use of imbibed seeds in cutting tests is strongly recommended. When assessing developing or soon-to-be-harvested crops, moisture content may be sufficient and thus, seed imbibition not required. In judging collection timing, one can allow cut seed to dry overnight—if the megagametophyte does not shrink from the seed coat, the seeds are ready to be collected (Eremko et al. 1989).

X-ray radiographs offer another method for visually assessing and preparing a permanent point-in-time record of a seedlot. X-rays are generally performed on seeds at storage moisture content between 5 and 10% as details about seed anatomy are clearly visible (Figure 11). Problems such as insect-infested seeds and cracks in the seed coat are readily visible on x-rays, but difficult to detect by other means. Film choices include options for instant development, great for immediate decision-making, or negative film for a higher quality, archival record of seedlot quality.

Figure 11 Examples of x-ray radiographs on a) seed at storage moisture content and b) soaked seeds at approximately 30% moisture content. The seed in the green ellipse (a) illustrates a viable seed; the seed in the white ellipse is a non-viable empty seed.

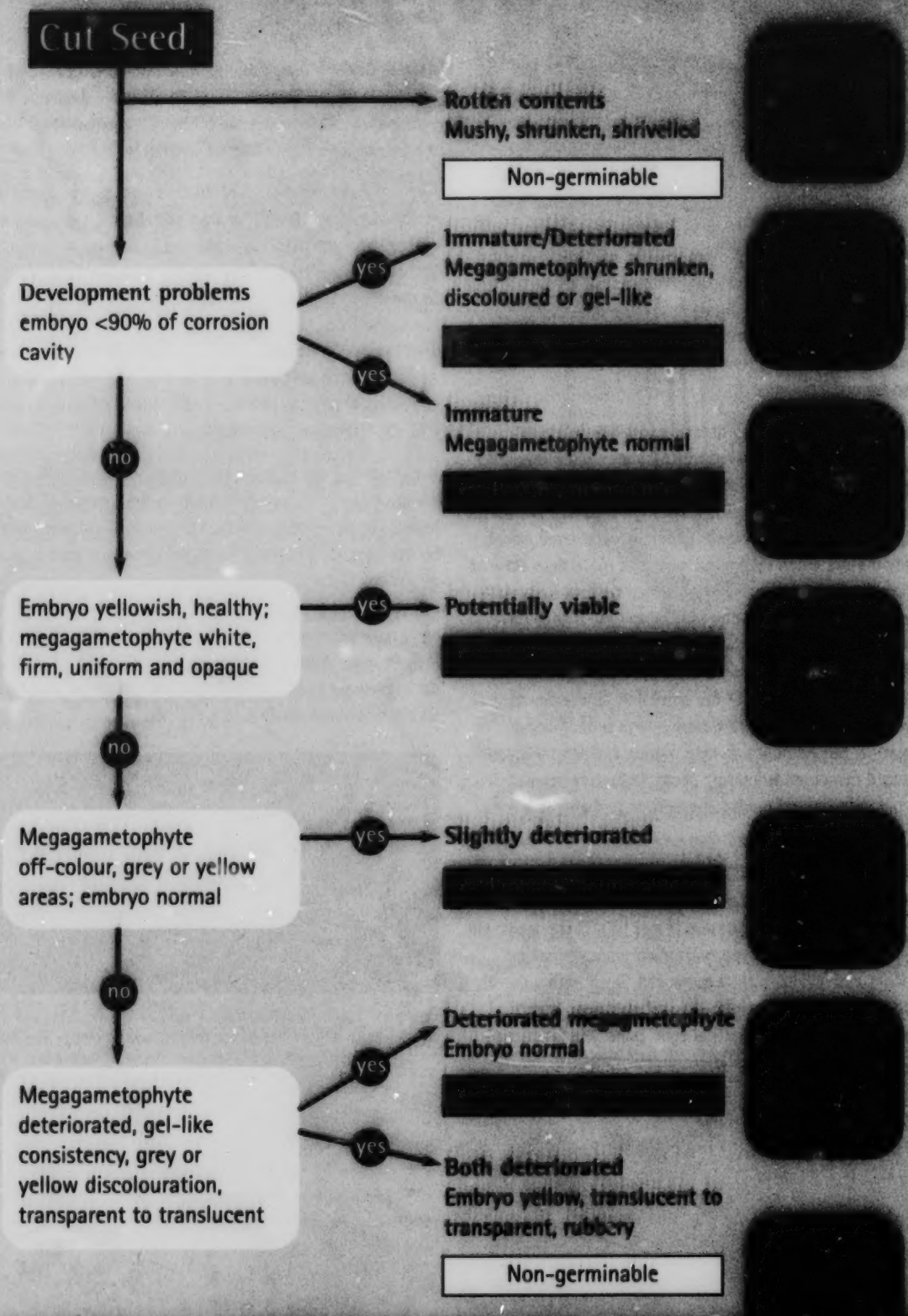
Cut Seed
Rotten contents
Mushy, shrunken, shrivelled
Non-germinable
Development problems
embryo <90% of corrosion cavity
yes
Immature/Deteriorated
Megagametophyte shrunken, discoloured or gel-like
yes
Immature
Megagametophyte normal
no
Embryo yellowish, healthy; megagametophyte white, firm, uniform and opaque
yes
Potentially viable
no
Megagametophyte off-colour, grey or yellow areas; embryo normal
yes
Slightly deteriorated
no
Megagametophyte deteriorated, gel-like consistency, grey or yellow discolouration, transparent to translucent
yes
Deteriorated megagametophyte
Embryo normal
yes
Both deteriorated
Embryo yellow, translucent to transparent, rubbery
Non-germinable

Figure 9

Figure 10

	Viable	Questionable Viability			Immature		Non-viable	
		EMBRYO						
	Good	Normal	Normal	Deteriorated translucent rubbery	Development problems	Development problems	Rotten	Comments/other
Seedlot/ Sample	# of seeds	MEGAGAMETOPHYTE						
		Good	Slight discolouration	Grey/Yellow deteriorated	Grey/Yellow deteriorated	Normal	Shrunken/ discoloured	Rotten	

Insects may attack conifer reproductive structures at any time from cone bud initiation through to cone collection and seed extraction. Insects that seriously damage cones and seeds are problematic, for the most part, during the pollination period and early cone development. However, some are likely to be encountered during cone collection and processing. This chapter discusses identification of these important insects and the practical significance of their actions to seedlots (Table 1, page 14). For information on other cone and seed insects, refer to Hedlin et al. (1980), Portlock (1996), and Turgeon and de Groot (1992). For insects of importance to conifer nurseries, see Hamm et al. (1990), Finck et al. (1990) or Sutherland et al. (1989).

Insects are natural, integral components of forest ecosystems. However, because of the human interest in wood products, many species of insects become our major competitors in the utilization of forest resources. Understanding the biology of these insects and learning how to live and deal with them is the challenge of forest insect management.

Forest insects that feed directly on conifer cones and/or seeds are termed **conophytes** (Turgeon et al. 1994) and are in direct competition with us for these resources. Only about 100 of the 50 000 species of insects known in Canada are conophytic. Of these conophytes, only a small number have an economic impact on our crops (de Groot et al. 1994). Although conifer hosts and conophytic insects coexist, certain conophytes can destroy entire cone crops in some years, particularly when crops are small following a previous year of large crops (de Groot et al. 1994). Conophytes can be readily divided into two categories. Obligate conophytes are those insects that *must* complete some part of their life cycle within conifer reproductive structures (Figures 12–14), while heteroconophytes (or facultative conophytes) do not require cones and

Obligate conophytes are those insects that must complete some part of their life cycle within conifer reproductive structures, while heteroconophytes do not require cones and seeds but feed upon them when they are available

Figure 12 Obligate conophytes within conifer cones – moth and cone gall midge: a) damage caused by larvae of Douglas-fir cone moth (black circle) and cone gall midge (white circles) in Douglas-fir cone; b) cone moth pupa (black circle) and damage (white circles) and cone gall midge larvae and damage (orange circle) in Douglas-fir cone; and c) moth pupa and damage in pine cone.

Figure 13 Obligate conophytes within conifer cones – seedworms and coneworms: a) ponderosa pine seedworm seed damage (white circle) and overwintering caterpillars in ponderosa pine cone; b) spruce seedworm seed damage (black circles) and overwintering caterpillars in interior spruce cone; c) coneworm caterpillar and damage in interior spruce cone; and d) ponderosa pine coneworm caterpillar, pupa and damage in ponderosa pine cone.

seeds but feed upon them when they are available (after de Groot et al. 1994, Turgeon et al. 1994).

Heteroconophytic insects are unlikely to impact the activities of seed handlers and are not dealt with in detail here. In British Columbia, western spruce budworm is a common and well-known heteroconophyte. Contorted, mature cones (especially of Douglas-fir or spruce) with some amount of surface damage *may* have been fed upon by western spruce budworm in early spring. Typically, it is impossible or difficult to extract seeds from such cones. Insect activity not only damages or destroys seeds but can reduce yield by limiting cone opening illustrating that losses from insects may be indirect through the reduction in seed yield.

Obligate conophytic insects are often host-species and host-cone tissue specific

Obligate conophytic insects are often host-species and host-cone tissue specific. For instance, the larvae of the Douglas-fir seed wasp are found only in Douglas-fir seeds (Figure 14c, d); larvae of a closely related species only in seeds of western and mountain hemlocks; larvae of the Douglas-fir cone gall midge only in Douglas-fir cone scales (Figure 12a, b). For many other examples, see Hedlin et al. 1980.

Natural stands of conifers often bear large cone crops in one year, then grow for several years producing few or no cones. Obligate conophytic insects have developed various biological strategies to survive the cone drought years and avoid over-exploitation of resources (see Turgeon et al. 1994). For seed and cone collectors and processing technicians, the most noticeable effect of these strategies is that obligate conophytes seem to disappear for several years only to re-appear unexpectedly in large numbers, often destroying an entire cone crop. This is especially true for cone crops in natural stands. In conifer seed orchards, which are normally managed to produce cone crops on a more regular basis, conophytic insects tend to be more consistently present and damaging (Finck et al. 1990).

In conifer seed orchards, which are normally managed to produce cone crops on a more regular basis, conophytic insects tend to be more consistently present and damaging

Figure 14 Obligate conophytes within conifer seeds: a) seed midge larva (circled) in *Abies* seed; b) seed midge (white circles) and seed wasp larvae (orange circle) in seeds in sub-alpine fir cone; c) radiograph showing three Douglas-fir seed wasp larvae (circled) in Douglas-fir seeds; and d) Douglas-fir seed wasp larva in seed in Douglas-fir cone.

The damage caused by obligate conophytic insects is often not readily apparent. For instance, ponderosa pine coneworm caterpillars may destroy the contents of conifer seed cones while leaving little or no external evidence of their activities (Figure 13d). Likewise, seeds destroyed by seed wasps are indistinguishable from undamaged seeds without dissection or the use of x-ray techniques (Figure 14c, d).

Because of these factors, cone crops must always be assessed for damage caused by conophytic insects. Preferably, this is carried out prior to harvest so that time and money are not wasted harvesting worthless crops. Insect assessments should also be carried out routinely at cone processing centres upon receipt of incoming cone crops or, at minimum, just prior to seed extraction. Refer to the first chapter in Portlock (1996) and Turgeon and de Groot (1992) for detailed discussions of pre-harvest cone crop monitoring, sampling, and damage assessment techniques.

Ideally, post-harvest assessments of cone crops should be based upon examination of 50 cones selected randomly from each seedlot. Operationally, due to the small size of some seedlots and timing constraints, random samples of 20 to 25 cones per seedlot are more practical and appear to provide satisfactory damage assessments.

Seed orchard seedlots usually have been subjected to rigorous assessment (typically involving dissection of 50 conelets per orchard) for the presence of economically important conophytes during the pollination period and through maturation. However, in seed orchards, certain conophytic insects are not routinely monitored (e.g., seed wasps) and others may become established or otherwise cause damage after the assessment (e.g., some coneworms and the western conifer seed bug). Therefore, it is important that all seedlots, regardless of origin, be assessed for insect damage at the processing centre prior to seed extraction.

While minimal post-harvest assessment includes the determination of average numbers of filled seed per cone (see chapter 1 in Portlock [1996] or Appendix 3), detailed assessment should also include identification and a tally of conophytic insects observed and, if feasible, some level of quantification of associated damage (e.g., estimate of percent of cones affected).

The following sections outline insect problems of direct relevance to conifer seed cone collection, and seed extraction and handling. For information on specific cone and seed insects, see Hedlin et al. (1980), and Furniss and Carolin (1980).

Moth Caterpillars in Stored Cones

Conophytic moth larvae (caterpillars – Figures 13, 15a) may continue feeding within stored cones until seeds are extracted. It is important to identify potential caterpillar problems and deal with them promptly to avoid unnecessary post-harvest loss of seeds. Caterpillars are the immature (usually actively feeding) stages of moths and butterflies. Prior to transforming into adult moths, caterpillars enter a dormant, usually inactive, pupal stage (Figures 12b, c; 13d; 15b). Seed handling technicians and cone collectors should be able to differentiate between moth larvae and pupae.

To differentiate between caterpillars and pupae, compare Figure 15a (caterpillar) and 15b (pupae) as well as the two life stages present in Figure 13d (caterpillar on surface of cut cone, pupa embedded in cone tissue). Caterpillars have a soft, cylindrical, elongated, and segmented body, a small but distinct head, three pairs of short walking legs near the head end, and a variable number of pairs of stubby prolegs nearer the hind end. Moth pupae are cylindrical but compact with a shiny covering and may or may not be enclosed in some sort of a cocoon. Close examination of a moth pupa with a handlens or microscope often will reveal legs, wings, antennae, and other appendages of the future adult, tightly bound up under the "skin" (Figure 15b).

Conophytic moth larvae may continue feeding within stored cones until seeds are extracted

Presence of caterpillar damage is indicated by bore holes (Figure 13d) or cavities on the surface of cones with associated frass and, often, webbing. Within cones, frass-filled tunnels through cone tissue (Figure 12b) indicate the presence of caterpillars. Many (but not all) conophytic moths overwinter within damaged cones as caterpillars or pupae. Efforts should be made to determine if pupae or caterpillars are present in stored cones. Pupae will cause no further damage to seeds but some types of caterpillars (especially coneworm caterpillars) may continue to feed.

Any brownish-coloured caterpillar, up to 15–20 mm long (Figures 13d, 15a) and found burrowing randomly through cone tissue (Figure 13c, d) at or post-harvest may be a coneworm caterpillar. Coneworms may occur in cones of virtually all BC conifers, especially Douglas-fir, true firs, pines, and spruces. In contrast, similar sized but whitish caterpillars found only in the cone axis and only in ponderosa pine (Figure 13a) and spruces (Figure 13b) are seedworm caterpillars. When found in mature cones, seedworm caterpillars have finished feeding and will cause no further damage to seeds.

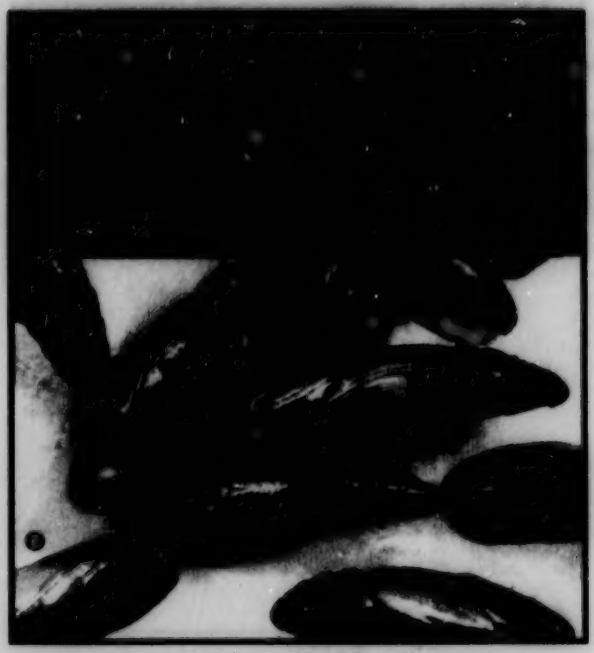

Figure 15 a) Fir coneworm caterpillar and b) pupae.

Stored conelots harbouring coneworm caterpillars should be kept cool, to slow feeding activity, and processed as quickly as possible to minimize further damage. Collect suspected coneworm caterpillars and send them alive (preferably) or preserved in ethanol to a cone and seed entomologist for identification.

Disposal of Insect-infested Cones

Whenever feasible, make an effort to identify damaging insects found in cones. Some conophytic insects found in harvested cones will survive within spent cones or associated debris after kilning and seed extraction (Ministry of Forests unpublished data). These insects can mature and emerge later to cause problems in future cone crops. Of particular concern are seedworm caterpillars in the axes of ponderosa pine (Figure 13a) and spruce (Figure 13b) cones, Douglas-fir cone gall midge larvae in Douglas-fir cone scales (Figure 12a), and any conophytic moth pupae (Figures 12c, d; 13d; 15b).

Note that larvae of spruce cone axis midges are often common in the axis of spruce seed cones (Figure 16) and may be confused with seedworm caterpillars. Spruce cone axis midges differ from seedworm caterpillars in being smaller, pinkish, legless, and enclosed within white, papery cocoons. Spruce cone axis midges apparently cause little or no damage to conifer seeds (Hedlin et al. 1980; Turgeon and de Groot 1992) and are not considered to be economically important.

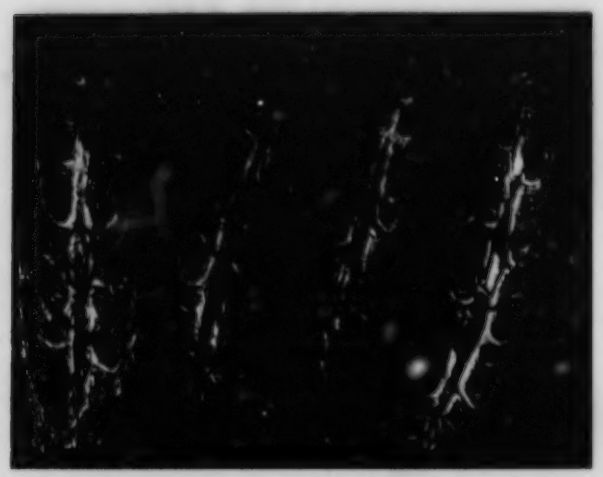

Figure 16 Spruce cone axis midge cocoons (circled) in interior spruce cones.

If possible, burn insect infested spent cones and processing debris (small amounts of material can be "cooked" in microwave or other ovens). This option may not be feasible for large-scale seed extraction facilities. In such cases, do not discard spent cones and debris in areas where future cone harvesting may occur. To avoid exporting insect problems to other areas, do not offer insect infested spent cones to landscapers, ornament dealers, or other commercial cone dealers.

Insects Within Seeds

Certain conophytic insects spend most of their lives entirely enclosed within, and consuming the contents of, otherwise healthy looking seeds. Adult seed wasps and seed midges lay their eggs on, in, or close to developing seeds in young cones (Hedlin et al. 1980). Larvae develop individually to maturity within seeds (seed wasp – Figure 14b, c, d; seed midge – Figure 14a, b) and overwinter there. The contents of infested seeds are completely consumed. Adult seed wasps and midges exit from seeds in spring, mate and lay eggs for a new generation of larvae. Until adult emergence, infested seeds show no external sign of insect inhabitants. Seed midge larvae are found in *Abies* and spruce seeds whereas seed wasp larvae are associated with most, if not all species of Pinaceae (de Groot et al. 1994, Hedlin et al. 1980).

Seed midges are not well known but seed wasps have been extensively studied. Seed wasps usually are present at some level in most uncleaned seedlots of susceptible conifers and may be responsible for considerable seed destruction. Some proportion of infested seeds may be removed during seed cleaning but levels of seed wasp or midge larvae in cleaned seedlots should always be determined (infested seeds will not germinate). This is easily carried out through standard radiography (Figure 14c) of a random sample of cleaned seeds (minimum 100 seeds). Alternatively, determine the presence of larvae in seeds by slicing open a random sample of seeds with scalpels or razor blades (see discussion on cutting tests in the chapter "Seed Condition").

Table 1 Scientific names of insects discussed in text

Common Name	Order	Family	Scientific Name
western conifer seed bug	HEMIPTERA	Coreidae	*Leptoglossus occidentalis* Heidemann
Douglas-fir cone moth	LEPIDOPTERA	Tortricidae	*Barbara colfaxiana* (Kearfott)
seedworm			*Cydia* spp.
ponderosa pine seedworm			*Cydia piperana* Kearfott
spruce seedworm			*Cydia strobilella* (Linnaeus)
coneworm		Pyralidae	*Dioryctria* spp.
fir coneworm			*Dioryctria abietivorella* (Grote)
ponderosa pine coneworm			*Dioryctria auranticella* (Grote)
Douglas-fir cone gall midge	DIPTERA	Cecidomyiidae	*Contarinia oregonensis* Foote
seed midge			*Dasineura* spp.
spruce cone axis midge			*Kaltenbachiola rachiphaga* (Tripp)
seed wasp (seed chalcid of others)	HYMENOPTERA	Torymidae	*Megastigmus* spp.
Douglas-fir seed wasp			*Megastigmus spermotrophus* Wachtl

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Seed wasp infested seeds should not be exported. Douglas-fir seed wasp was introduced to Europe in seedlots imported from Canada early in the 20th century. Currently, regeneration of Douglas-fir in Europe is seriously hampered because of high levels of seed destruction attributable to this insect. Seed wasp levels in cleaned Douglas-fir seedlots can be reduced by using the "incubation drying separation" (IDS) method described by Sweeney et al. (1991) or through some type of specific gravity separation (see discussion in "Seed Processing"). Operationally, gravity table extraction is likely to be a more practical method than IDS to reduce seed wasp levels in large seedlots.

Identifying Western Conifer Seed Bug Damage

Western conifer seed bugs insert their syringe-like mouthparts deep into cones and feed upon developing and mature seeds during the growing season. The seed coat is left undamaged but seed contents are partially or completely consumed (Figure 17), or seeds may fuse to scales, especially when seed bug feeding has occurred early in seed development. Accurate identification of seed bug damage in extracted seeds is very difficult (Bates 1999; Bates et al. 2000). Damage is not visible externally on fed-upon seeds, and internal damage (revealed through seed dissection or radiography) may have been caused by weather, poor pollination, or other environmental factors rather than seed bug feeding. Partially depleted seed contents are likely to be the result of seed bug feeding but it is not possible at present to determine the cause of total depletion. Researchers at Simon Fraser University have developed a marker that will accurately identify the presence of western conifer seed bug saliva within seeds (Lait et. al 2001). In the near future we hope that this tool will enable seed technicians to assess seed bug damage with a high degree of accuracy.

Accurate identification of seed bug damage in extracted seeds is very difficult

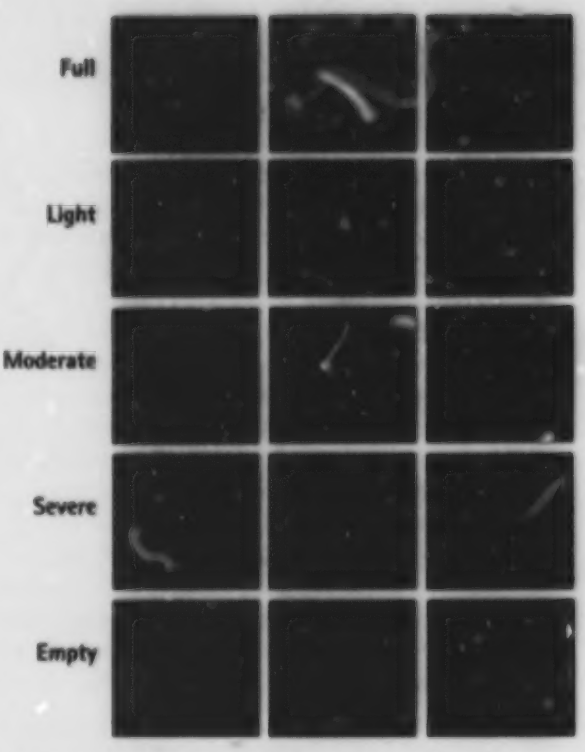

Figure 17 Radiographs of Douglas-fir seeds exposed to feeding by western conifer seed bug during cone development. Damage categories are: Full (no apparent damage), Light (>2/3 of seed contents remaining), Moderate (1/3–2/3 remaining), Severe (<1/3 remaining), and Empty (no contents remaining). Copied with permission from Bates (1999).

Fungi have been associated with seed and have evolved mechanisms for seed-borne transmission of diseases for more than 130 million years (Buller 1950). This long association between fungi and seeds is important because it indicates that, for certain pathogens, there has been a long period of evolutionary development leading to sophisticated host-pathogen relationships. Understanding these complex relationships is essential, as it is often difficult to isolate different causes of disease when control measures are developed and implemented (Maude 1996). The introduction of "exotic" pathogens in the absence of co-evolutionary development between host and pathogen can lead to significant and unchallenged damage to the host. This is especially important in intensively managed agricultural and forestry operations where seeds encounter new fungi while being handled in unnatural environments where they have not had time to evolve any natural defense mechanisms.

Seed-borne fungi are defined as those "that are dispersed in association with some kind of dispersal units of the host (i.e., seeds)" (Ingold 1953). This definition includes all seed types and all associated microfungi and is the one we will adopt. Some authors classify fungi as being either seed-borne or seed-transmitted (Thomsen and Schmidt 1999). They define seed-borne fungi to include all fungal types contaminating the surface of seeds or infecting seed tissues. Seed-transmitted fungi are those that cause no infection to a seed itself but infect seedlings in the nursery or field (Neergaard 1979). For the purposes of this guide, we are most interested in seed-borne pathogens and seed-borne diseases. Seed-borne pathogens (as opposed to diseases) are defined here as organisms which, whether on or in seeds, may or may not cause infections and symptoms on the seeds. Seed-borne pathogens associated with conifer *seeds* may inhabit the external or internal tissues of seeds. Seed-borne diseases occur on *seedlings* as a result of pathogens carried in or on the seeds, susceptibility of the host plant, and suitable environmental conditions.

Seed-borne fungi are dispersed in association with some kind of dispersal units of the host (i.e., seeds)

Seeds harbouring fungi can be described as being either contaminated or infected. Contamination is used to denote the occurrence of a pathogen as either spores or mycelium on the surface of seeds. Contamination may be entirely superficial where spores or mycelium are usually retained in small cracks or fissures in the seed coat. Infection refers to the penetration of seeds by an organism followed by the establishment of a relationship (i.e., saprophytic or parasitic) within the seeds. Once established, such a relationship can give rise to outward hyphal growth from within the seeds. While this hyphal growth can appear as a contaminant, it is indicative of the presence of an infection deeper within the seeds. In certain situations it may be possible to disinfest seeds that are only superficially contaminated. Once seeds have become infected by a fungus, it cannot be disinfected in this manner and control becomes more difficult.

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Seed Contamination/ Infection Routes

Seeds can become infected directly by a systemic invasion via mother plant tissues to the seed embryo (Figure 18a). Infection occurs this way through the xylem tissues of the mother plant to the embryo. This manner of seed infection is confined primarily to viruses and bacteria. However, some fungi infect seeds this way as well. A more common fungal infection sequence to the seed embryo is indirect, via the plant stigma or with conifers, as fungal hyphae grow from spores caught within receptive cones at or near the time of pollination, infecting the embryo (Figure 18b). Infection of seeds in this manner occurs only when the release and dispersal of fungal spores coincides with pollination. A species of *Botrytis* responsible for anther mould of red clover infects seeds in this manner (Silow 1934). In the interior of BC, cones of spruces can become infected in this manner by inland spruce cone rust (*Chrysomyxa pirolata* Wint.). Species of *Fusarium* may infect and become seed-borne in this way also.

Other common indirect infection routes in seeds are those of infection via flower or fruit parts to the ovary and ovule tissues or direct contact between seeds and

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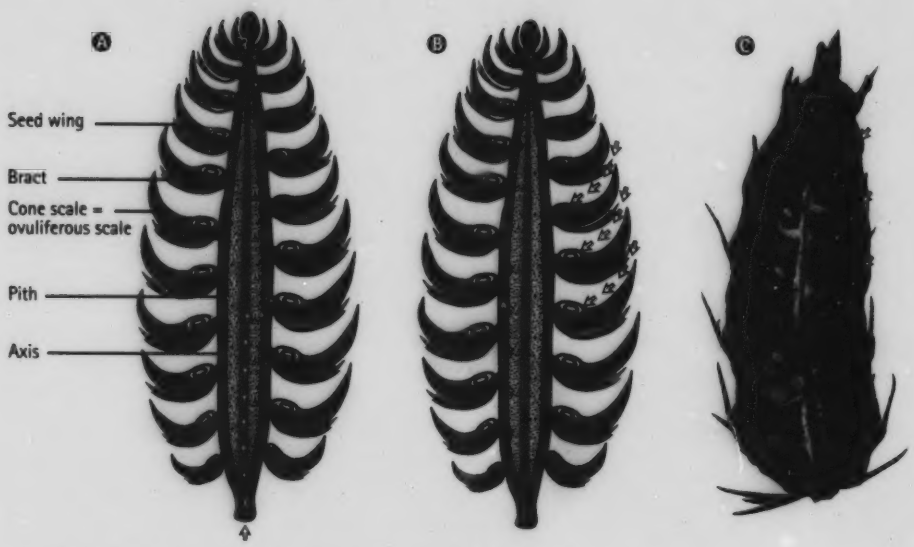

Figure 18 Direct and indirect infection routes (arrowed): a) direct invasion via the xylem of mother plant tissues; b) indirect invasion by spores at time of pollination; and c) indirect penetration of mature cone.

contaminated soil or water (Figure 18c). The so-called "seed" or "cold" fungus, *Caloscypha fulgens*, as well as *Sirococcus conigenus* become seed-borne in this manner. These latter two infection or contamination methods are the most common for conifer seeds. Seeds within cones can become contaminated when contacted by air-borne spores, air-borne water droplets containing spores, or by contacting contaminated soil directly. This can occur when cones are open and receptive during pollination or later when cones are mature and closed (Figure 18b, c). In each of these situations, seeds become contaminated as spores become lodged within small cracks and irregularities on their surface. Infection then follows as the spores germinate, penetrate, and invade the seeds. Seeds contacting contaminated soil may themselves become contaminated in a similar manner. Infection may also occur as spores or mycelium in the soil contact and penetrate the seed surface.

Pathogenic and Non-pathogenic Seed-borne Fungi

Many fungi are routinely found on conifer seeds, some of which are potentially pathogenic given the right environmental conditions, while others are relatively harmless. Also, some fungi may be pathogenic on seeds of low quality (i.e., damaged or immature), but cause no damage on healthy, vigorous seeds. Seed-borne organisms and for our purposes, fungi, are not all pathogenic nor do they pose a threat to seeds or subsequent seedling health. In fact, some fungi may be symbionts which are beneficial to the plant (Mallone and Muskett 1997). This is important because although the appearance of mould or fungal hyphae growing from seeds may indicate good conditions for fungal growth in general—a reflection of sub-optimal storage—it does not necessarily indicate the seeds to be inferior. Some saprophytic fungal species may produce toxic substances that control certain active pathogens (Mallone and Muskett 1997). A species of *Trichoderma*, known to produce antagonistic toxins, is used as a seed dressing to control damping-off due to seed- and soil-borne *Fusarium* species.

In BC, three fungal genera found on conifer seeds are of special importance to seed and seedling health. Species of *Fusarium* contaminate seeds and are responsible for damping-off of seedlings and potentially lead to *Fusarium* root rot and possibly *Fusarium* shoot blight. Both *Caloscypha* and *Sirococcus* infect seeds. *Caloscypha* is responsible for killing seeds while *Sirococcus* can kill the resulting germinants, and spread by spores to further infect and kill adjacent seedlings. These three important seed-borne fungi as well as other commonly occurring fungi found on BC conifer seeds are listed in Table 2 in order of decreasing seriousness to seed and seedling health.

Fusarium species

Species of fungi belonging to the genus *Fusarium* are responsible for both pre- and post-emergence damping-off and can be implicated with root rot and shoot blight of conifer seedlings (Bloomberg 1971; Bloomberg 1981; Nelson et al. 1981). *Fusarium* is primarily spread by spores borne by either air, water, soil, or seeds. Soil-borne *Fusarium*, which can overwinter as chlamydospores in soil or be introduced as spores by either air or water, is primarily a concern in bareroot nurseries. However, similar mechanisms to those encountered in natural soils occur in container settings where contaminated container growing media are encountered or when *Fusarium* spores are introduced by air or water (Figure 19). In these situations *Fusarium*-contaminated growing media can result in infected seedling roots leading in most cases to post-emergence damping-off, *Fusarium* root rot, or shoot blight, in this order of importance. Seed-borne *Fusarium* can lead to any of these results but is most often responsible for pre-emergence damping-off.

Life history

When and how conifer seeds become contaminated with *Fusarium* remains unclear. *Fusaria* are a ubiquitous group of fungi with spore inoculum present in the environment throughout the year. General disease cycles of pre- and post-emergence damping-off as well as *Fusarium* root rot and shoot blight illustrate possible times throughout the year when spores might be released and become available as contaminants (Figure 19). Seed-borne contamination may occur through indirect routes such as via cone parts to the ovary and ovule tissues or through direct routes when seeds contact contaminated soil and water. Dirty equipment can also contaminate seeds during interim storage, cone and seed processing, seed stratification, or at the nursery from contaminated sowing equipment, growing containers, or pallets. As *Fusarium* spores can be released throughout the year, at almost any time in the general life cycle of major BC commercial conifer seedlings, seeds are exposed to contamination over a wide range of conditions. Examination of tree seed samples from over 2600 seedlots stored at the BC Ministry of Forests Tree Seed Centre has indicated the frequency of seed-borne *Fusarium* to be the same on seeds originating from seed orchards and those taken from natural stands (Peterson 2000). *Fusarium* spores released from soil or grasses within and around seed orchards may be spread by irrigation sprinklers. This problem could be compounded by the use of sprinklers to control pollination in the spring. Indirect contamination through cone parts to the ovary and ovule tissues such as this could similarly occur in wild stands via rainfall. Seeds and cone parts harbouring *Fusarium* can contaminate processing facility equipment, contributing to further contamination of otherwise clean seeds. Regardless of the initial source, seed-borne *Fusarium* can spread throughout a contaminated seedlot during the period of imbibition prior to seed stratification. A general understanding of *Fusarium* disease cycles and potential times of contamination and spread presents opportunities for intervention during the pre- and post-collection phases of the seed handling system.

Fusarium is primarily spread by spores borne by either air, water, soil, or seeds

Table 2 Commonly occurring fungi found on BC conifer seeds in order of *decreasing* significance to seeds and seedling health

Seed-borne fungi	Fungus source and effects on seeds or seedlings
Fusarium spp.[a]	soil-, air-, and water-borne damping-off fungus
Caloscypha fulgens[a]	soil-borne pathogen kills and mummifies seeds
Sirococcus conigenus[a]	water-splash, air-borne from seedlings of infected seeds
Cylindrocarpon destructans	soil-borne saprophyte and weak parasite
Alternaria spp.	air-borne saprophyte and weak parasite
Phoma glomerata	soil-borne blight fungus
Phomopsis spp.	water-borne blight fungus
Botrytis cinerea	air-borne saprophyte and weak parasite
Trichoderma viride	soil-borne fungal antagonist
Penicillin spp.	air-borne saprophyte
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[a] Seed-borne fungi of greatest concern, whose presence is routinely tested for in BC.

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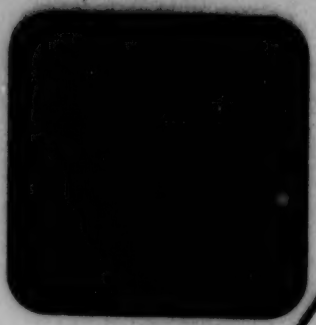
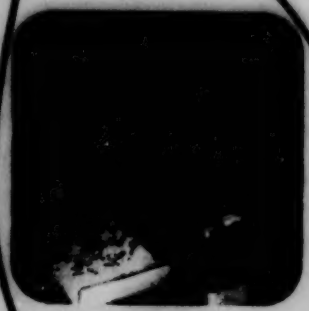


Ⓐ Pathogen overwinters or survives other adverse conditions as chlamydospores in small pieces of roots or organic matter in the soil.

Ⓑ In spring, spores contaminating seed or in proximity to germinants or young seedlings, germinate and infect host.



Ⓒ **Post-emergence damping-off:** Stem rotting at the groundline and subsequent toppling of shoot; seedlings are susceptible up to 6 weeks after emergence; disease spreads by mycelium or spores.

Ⓓ **Pre-emergence damping-off:** Rots seeds or germinants; Disease spreads by mycelium or spores.

Fusarium root rot: In early summer chlamydospores germinate and infect seedling roots that grow nearby. Later in season when seedlings are larger, more crowded and stressed, fungus ramifies and destroys root system.

Fusarium top blight: Needle chlorosis, browning and desiccation with hook-shaped leader tip. Fruiting structures on stem tissue can release spores to contaminate and infect other tissues.

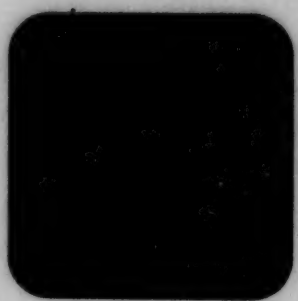


Figure 19

...the frequency of seed-borne Fusarium *is the same on seeds originating from seed orchards and those taken from natural stands*

Sources of contamination

Probable sources of seed contamination by species of *Fusarium* are indicated in Figure 20. Seeds may become initially contaminated with *Fusarium* both prior to entering the seed handling system as well as at several points within the system. Seed orchard seeds, as well as those originating from wild stand collections, are susceptible. Regardless of the initial source, the incidence of *Fusarium* can intensify within a contaminated seedlot depending on how the seeds are handled.

Caloscypha fulgens

The common names "seed" or "cold" fungus attached to this pathogen refer to its seed-borne nature as well as its ability to spread from diseased to healthy seeds during conditions of cold, such as stratification. This fungus was first reported in Ontario bareroot nurseries where it caused damping-off of fall-sown pine seeds (Epners 1964). It was next identified as a pathogen in Britain on Sitka spruce seeds that had been imported from North America (Salt 1971). Salt (1971) described and named the fungus *Geniculodendron pyriforme* Salt. This is the asexual form of the fungus and its sexual or "perfect" state was labeled *Caloscypha fulgens* in 1978 (Paden et al. 1978). The fungus was subsequently isolated from stored seeds in BC (Sutherland 1979), Oregon, and Washington (Harvey 1980). This pathogen becomes seed-borne when cones contact forest duff or litter where *C. fulgens* lives.

Life history

The seed fungus inhabits forest duff. It can infect cones through direct contact with forest soil during collection from wild stands (Figure 21). In spring, usually after snowmelt, *C. fulgens* growing in the forest duff produces cup-shaped, orange fruiting bodies called **ascocarps**. Ascocarps produce sexual spores called **ascospores** while non-sexual components of the fungus can lead to the formation of **conidiophores** that produce asexual spores called **conidia**. Neither sexual nor asexual spores appear to play any role in seed infection but rather are responsible for disseminating the fungus. Seeds in cones become infected with *Caloscypha* when they contact mycelium or hyphal threads of the fungus growing in the duff. Incidence of diseased seeds is dependent on several factors, the primary one being the length of time cones remain in contact with the ground during cool moist conditions. Sutherland et al. (1989) point out that the optimum temperature for growth of the seed fungus is 20°C. However, appreciable growth occurs even at 1 or 2°C. The pathogen can spread among contaminated seeds during stratification where conditions are commonly cool and moist. Because infection within a seedlot can intensify in this manner, detection of low levels of infection is important. Avoiding prolonged cool, moist conditions such as those encountered during stratification, is central to management of the disease. Most species require stratification for optimal germination and one must critically examine the trade-off between decreased disease incidence and decreased germination capacity before abandoning stratification. However, *C. fulgens* infects and kills seeds, with their contents becoming hard and mummified rather than rotten, as is the normal result of damping-off. *Caloscypha fulgens* does not infect emerging seedlings once the seeds have germinated.

Sources of contamination

Caloscypha infects seeds after cones contact the forest duff in areas where the fungus occurs. This largely limits the occurrence of seed-borne *Caloscypha* to seeds originating from wild stand collections. The initial source of *Caloscypha* is encountered both prior to and just after seeds enter the seed handling system; however, infection can spread to healthy seeds at several points within the system (Figure 22).

Sirococcus conigenus

Sirococcus conigenus causes a shoot blight of over 19 coniferous species in North America, Europe, and Asia (Hamelin 1986). The disease is particularly severe in BC forest nurseries where it mainly affects spruce, lodgepole pine (Illingworth 1973; Sutherland and Van Eerden 1980; Sutherland et al. 1981; Sutherland et al. 1982), and western hemlock (Funk 1972). Recent unpublished data indicates *S. conigenus* also has a large impact on ponderosa pine (*Pinus ponderosa* Dougl. Ex P.&C. Laws) (J. Dennis, pers. comm., May, 2000). In BC where the disease is known to be seed-borne on spruces, *S. conigenus* has recently been observed on western larch seeds (Peterson 1998, unpubl.[2]).

Life history

Sirococcus conigenus is confirmed to be seed-borne on spruce and can create disease centres which develop from infected germinants originating from infected seeds (Figure 23). Fully developed seeds in cones become infected. However, the mode of infection is unknown. The fungus produces spores in fruiting structures called **pycnidia**. Water plays a significant role in spore dissemination and it is likely rain-splashed spores that infect cones. Disease also results as air- and mist-borne

[2] M.J. Peterson, unpublished data, 1998, BC Min. For., Tree Seed Centre, Surrey, BC.

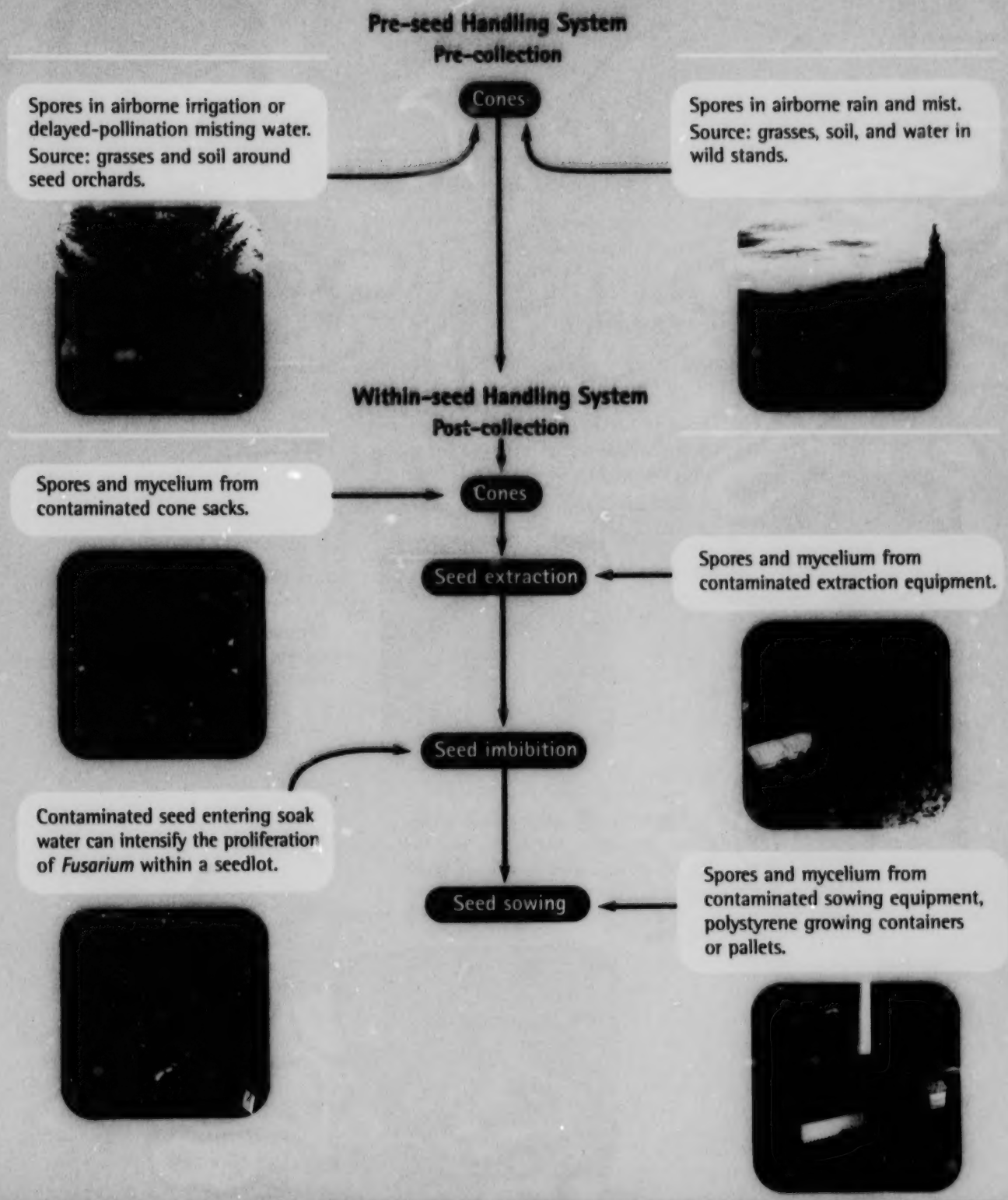

Pre-seed Handling System
Pre-collection
Cones
Spores in airborne irrigation or delayed-pollination misting water.
Source: grasses and soil around seed orchards.
Spores in airborne rain and mist.
Source: grasses, soil, and water in wild stands.
Within-seed Handling System
Post-collection
Spores and mycelium from contaminated cone sacks.
Cones
Seed extraction
Spores and mycelium from contaminated extraction equipment.
Seed imbibition
Contaminated seed entering soak water can intensify the proliferation of Fusarium within a seedlot.
Seed sowing
Spores and mycelium from contaminated sowing equipment, polystyrene growing containers or pallets.

Figure 20

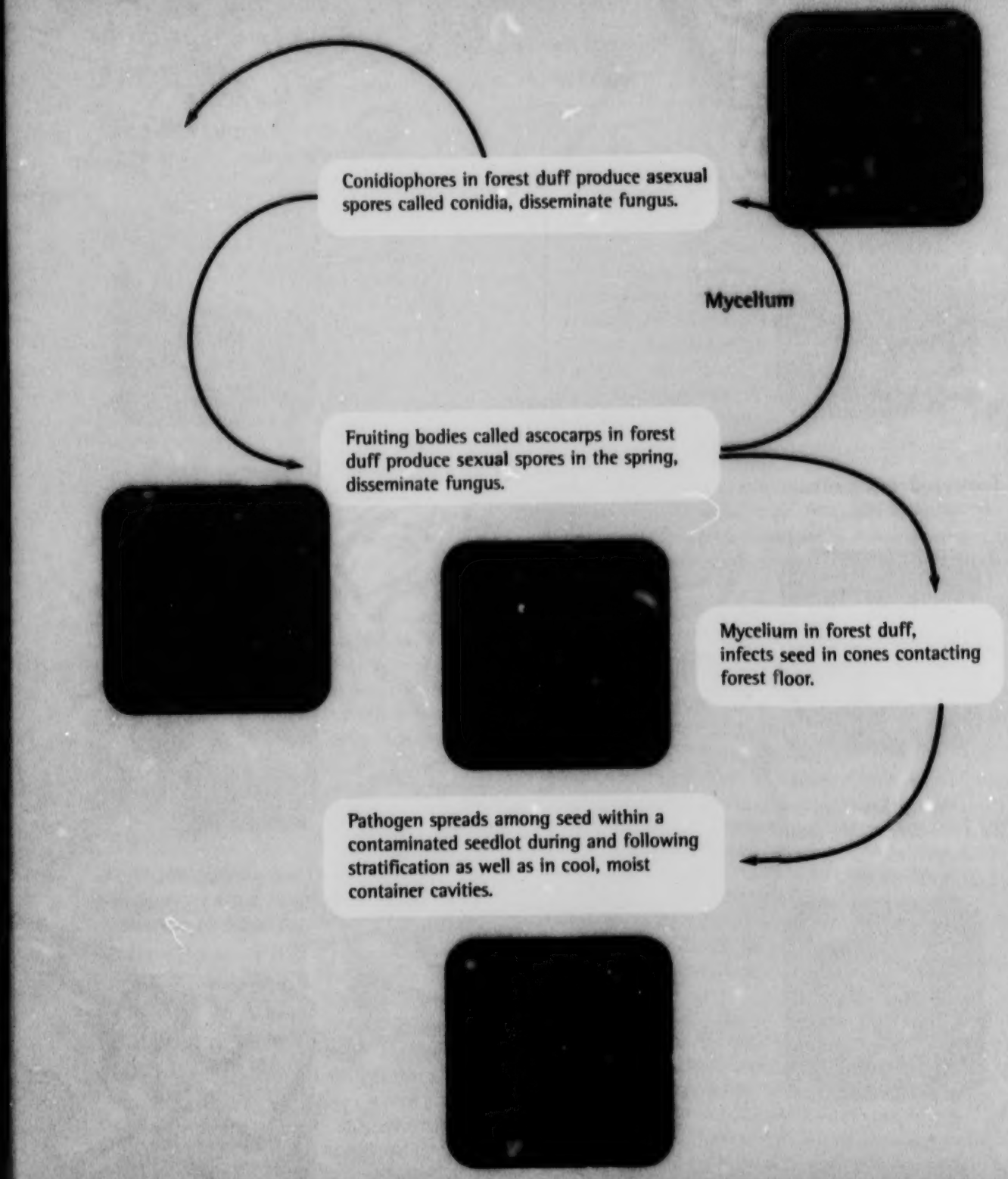

Conidiophores in forest duff produce asexual spores called conidia, disseminate fungus.
Mycelium
Fruiting bodies called ascocarps in forest duff produce sexual spores in the spring, disseminate fungus.
Mycelium in forest duff, infects seed in cones contacting forest floor.
Pathogen spreads among seed within a contaminated seedlot during and following stratification as well as in cool, moist container cavities.

Figure 21

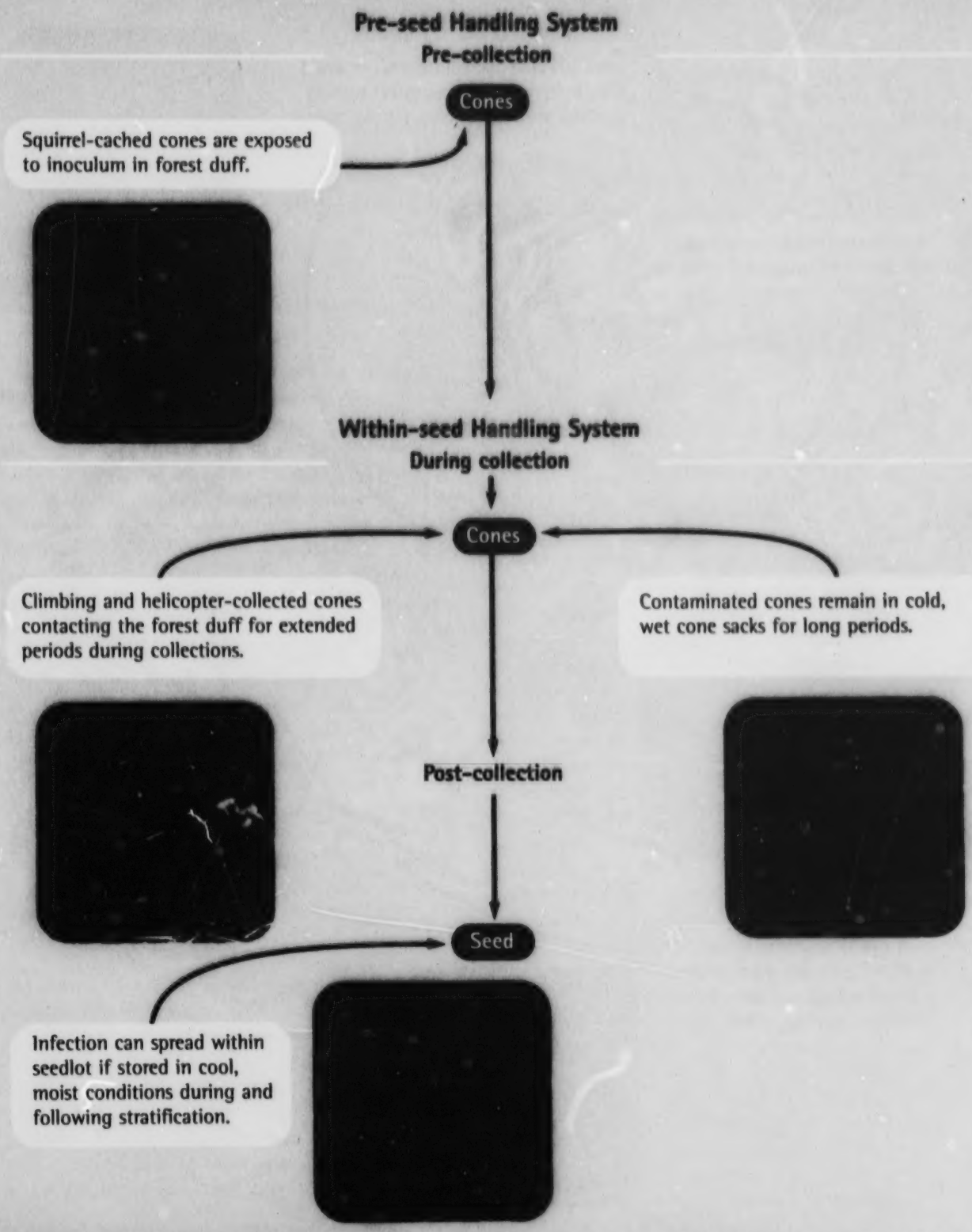
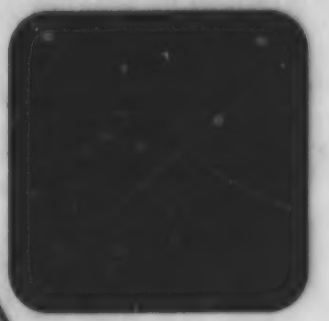

Pre-seed Handling System
Pre-collection
Cones
Squirrel-cached cones are exposed to inoculum in forest duff.
Within-seed Handling System
During collection
Cones
Climbing and helicopter-collected cones contacting the forest duff for extended periods during collections.
Contaminated cones remain in cold, wet cone sacks for long periods.
Post-collection
Seed
Infection can spread within seedlot if stored in cool, moist conditions during and following stratification.

Figure 22

Rain-splashed pycnidiospores dispersed from infected wild trees infect nursery seedling shoots and cones in the spring.



Pycnidia on nursery seedlings release pycnidiospores.

Pathogen overwinters in dead tissue in and around nursery. Pycnidiospores are produced and released in spring.

Pycnidiospores on cones germinate and infect seeds.

Seed-borne inoculum results in diseased seedling which can produce pycnidiospores and spread to other seedlings.

Figure 23

spores enter from outside nurseries and although this is usually secondary to seed-borne sources, it is implicated with the appearance of *Sirococcus* blight on lodgepole pine seedlings (Peterson 1996). The disease is favoured by frequent or prolonged periods of below-average temperatures and wet, cloudy weather. Spruce seeds are the primary species assayed for this fungus both for its ability to kill spruce seedlings and also for the ability of infected spruce seeds to become a potential inoculum source for lodgepole pine seedlings.

Fully developed seeds in cones become infected with Sirococcus conigenus. *However, the mode of infection is unknown*

Infection on spruce seedlings occurs most readily between temperatures of 16 and 21°C, in the presence of moisture, and under low light conditions (Wall and Magasi 1976). Diseased seedlings exhibit a tip dieback as well as stem and branch cankers in the current years growth. Foliage distal to the infection becomes yellow and dies. Smaller seedlings are killed from multiple infections, while larger stock become forked and misshapen. Nursery losses are due to seedling death as well as from culling misshapen stock. In BC, the most common form of damage on container seedlings is the death of the primary needles from the base upwards. *Sirococcus conigenus* is seed-borne on spruce (Sutherland et al. 1981) and unlike most other seed-borne diseases, seedlings can germinate from these seeds. Pycnidia may then form on needles of the infected germinant (Figure 23). As spores are released and dispersal progresses, the disease can spread outward from a single seedling to infect others. This allows the disease to spread from container cavities sown with single or multiple seeds, to other seedlings in the nursery. It is this ability to spread to other seedlings from a single infected seed that makes early detection of the presence of seed-borne *Sirococcus* especially important.

Sources of contamination

Rain-splashed *Sirococcus* spores result in infection of cones. Seeds in contaminated cones become infected at this point, prior to entering the seed handling system (Figure 24). This usually occurs in wild stands, although seed orchard trees are not immune to infection. Exposure to additional *Sirococcus* inoculum occurs within and beyond the seed handling system with seeds as well as seedlings being susceptible.

It is this ability to spread to other seedlings from a single infected seed that makes early detection of the presence of seed-borne Sirococcus *especially important*

It is important to cull seedlings infected with *Sirococcus* and burn them to destroy any fruiting bodies on the dead foliage, which can otherwise continue to release spores. Dead seedlings should not be left to overwinter as pycnidia can form and release spores again in the spring. Finally, as spores can spread some distance via rain splash and mist, whenever possible, lodgepole pine seedlings should not be grown downwind of spruce crops known to have high *Sirococcus* infection levels.

Not all species of conifers are affected by each of the three major seed-borne fungi outlined above. Most seeds are susceptible to *Fusarium* contamination, fewer species become infected with the cold fungus and fewer still by *S. conigenus*. The conifer species on which each of the above seed-borne fungi has been found to occur are shown in Table 3.

Laboratory Testing for Seed-borne Pathogens

Establishing Testing Priorities

The potential for conifer seeds to become contaminated or infected with species of *Fusarium, Caloscypha fulgens*, or *Sirococcus conigenus* makes testing for their presence a viable first step for managing these seed-borne fungi. Different species are susceptible to contamination or infection from each pathogen in varying degrees. Facts such as the ability of any of the fungi to spread within a seedlot, ways in which the seeds are collected, and the fact that some tree seeds are not affected by any of these fungi, result in some species being more susceptible than others. Also, if attacked, seeds from some species represent a higher potential monetary loss. For these reasons, seeds are tested for fungal pathogens in order of priority based on each species' potential to become contaminated or infected by each pathogen. Past testing has indicated the frequency with which individual species have been contaminated or infected by each species of fungi (Figures 25, 26, 27 [page 29]) and these data, combined with known information about each fungus' life history, the value of the seeds, and incidence of disease occurring on seedlings, are used in deciding priorities for testing. Seed-borne fungi can also influence seedling health with tree species most susceptible to seed-borne fungi also tending to be most affected by seedling disease (Figures 28, 29 [page 29]). A synthesis of this conifer and fungal information is presented in a matrix of seed testing priorities (Table 4).

Seed Testing Methods

In BC, screening tests called fungal assays are routinely conducted for the presence of contaminated or infected seeds within

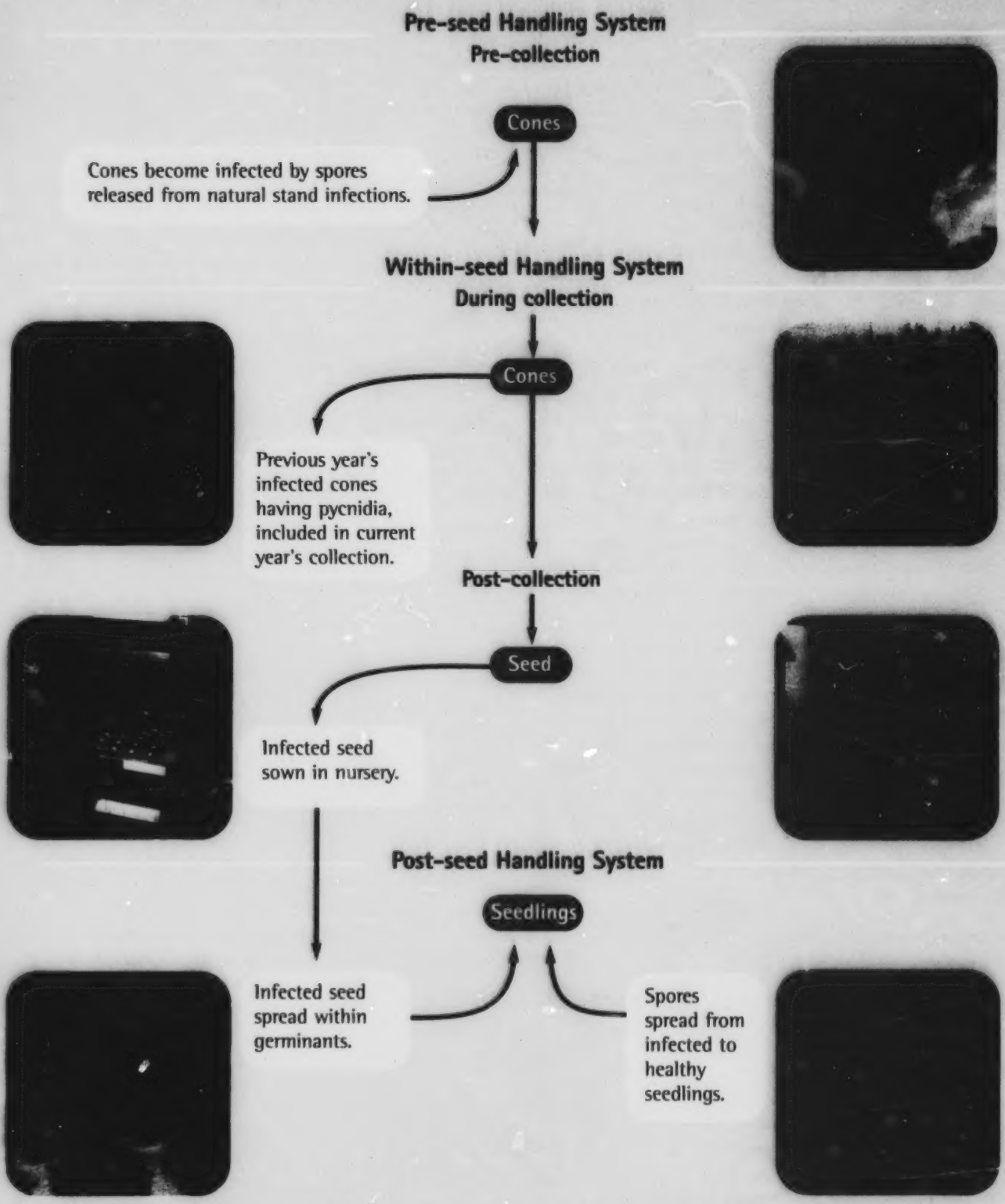

Pre-seed Handling System
Pre-collection
Cones
Cones become infected by spores released from natural stand infections.
Within-seed Handling System
During collection
Cones
Previous year's infected cones having pycnidia, included in current year's collection.
Post-collection
Seed
Infected seed sown in nursery.
Post-seed Handling System
Seedlings
Infected seed spread within germinants.
Spores spread from infected to healthy seedlings.

Figure 24

Table 3 Tree species affected by seed-borne *Fusarium* spp., *Caloscypha fulgens*, and *Sirococcus conigenus* in decreasing order of frequency as indicated through fungal assays

	Fusarium spp	*Caloscypha fulgens*	*Sirococcus conigenus*
Affected tree species	Interior Douglas-fir	Sitka x interior spruce hybrid	Sitka x interior spruce hybrid
	Western larch	Grand fir	Western larch
	Western white pine	Subalpine fir	Sitka spruce
	Western redcedar	Interior spruce	Interior spruce
	Ponderosa pine	Sitka spruce	Western hemlock
	Coastal Douglas-fir	Western white pine	
	Sitka x interior spruce hybrid	Noble fir	
	Grand fir	Amabilis fir	
	Western hemlock	Interior Douglas-fir	
	Subalpine fir	Western hemlock	
	Sitka spruce	Coastal Douglas-fir	
	Yellow-cedar		
	Noble fir		
	Amabilis fir		
	Interior spruce		
	Mountain hemlock		
	Interior lodgepole pine		

seedlots stored at the Tree Seed Centre. The assays are carried out following a strict set of protocols, providing confidence in the results from year to year (repeatability) as well as between testing agencies and laboratory personnel. Specific assay protocols are described and rationales are provided for each test.

Before the fungal assay protocols were established, studies looking at suspect seedlots were conducted on dry unstratified seeds. For this reason as well as the potential for *Fusarium* or *Caloscypha* to spread within seedlots during and following stratification, assays are conducted on dry unstratified seeds. This also simplifies seed handling and reduces variability in seed condition prior to testing. Seeds are withdrawn from the Tree Seed Centre and stored at 4°C after which testing is carried out as expeditiously as possible.

Past testing combined with known information about each fungus' life history, the value of the seeds, and incidence of disease occurring on seedlings, are used in deciding priorities for testing

Derivation of Seed Sample Size

Small samples of seed from specific seedlots can be assayed to determine the rate of contamination or infection for an entire seedlot. It is important that random, representative samples of appropriate size are chosen to allow these inferences to be made with what is considered an acceptable degree of certainty. Sample size is determined based on several factors including the level of contamination to be detected, the expected variation within the sample, and the amount of risk associated with our inferred estimate for an entire seedlot. Knowledge of each organism's life history and some historical information regarding its frequency of occurrence is also used to help establish the size of sample to be assayed. Sample sizes given are not adjusted for seedlot size, but sampling intensity is, according to ISTA (1999) standards.

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	Coastal Douglas-fir	Western white pine	
	Sitka x interior spruce hybrid	Noble fir	
	Grand fir	Amabilis fir	
	Western hemlock	Interior Douglas-fir	
	Subalpine fir	Western hemlock	
	Sitka spruce	Coastal Douglas-fir	
	Yellow-cedar		
	Noble fir		
	Amabilis fir		
	Interior spruce		
	Mountain hemlock		
	Interior lodgepole pine		

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Table 4 Seed testing priority[a] for fungal pathogens by conifer species

Tree species	Fungal species		
	Caloscypha fulgens	*Fusarium* spp.	*Sirococcus conigenus*
Ba	**H**	M	L
Bg	M	M	L
Bl	**H**	**H**	L
Cw	L	M	L
Fdc	L	**H**	L
Fdi	L	M	L
Hw	L	M	L
Lw	L	**H**	M
Plc	L	L	L
Pli	L	L	L
Pw	M	**H**	L
Py	L	**H**	L
Ss	**H**	M	**H**
Sx	M	M	**H**
Sxs	M	M	**H**
Yc	L	L	L

[a] L = low priority, M = medium priority, **H** = high priority.

Average levels of *Fusarium* are typically less than 2.5% (Figure 25). Past sampling also indicates a moderate degree of variation within seedlots. Occasionally seedlots having contamination levels higher than this are encountered, but these are rare. All species of *Fusarium* are not pathogenic and those that are, are often only weakly so. Therefore, it is currently desirable to detect *Fusarium* levels within any seedlot at a relatively conservative level of 5%. With this knowledge and the desire to detect levels of *Fusarium* with a 95% degree of confidence, it is necessary to sample 500 seeds per seedlot.

Assays for *Caloscypha* have shown average infection levels to be about 3% (Figure 26) but the amount of variation between species is higher than for *Fusarium*. With this in mind, detecting levels of *Caloscypha* contamination of 5% but with a greater allowance for variation around this estimate, has indicated a sample size of 250 seeds to be sufficient.

Seed-borne *Siroccocus* has the ability to spread systematically within an infected germinant and spread via spores to infect adjacent seedlings. *Sirococcus* is primarily seed-borne on spruce seeds but can spread to other seedlings. Assays for *Siroccocus* have shown average infection levels to be less than 1% (Figure 27). Therefore, it is desirable to detect low levels of infection within a seedlot. We want to detect *Sirococcus* at infection levels of 1% within a seedlot, which requires a sample size of 1500 seeds.

Laboratory Assay Methods

Fusarium species

Seeds become contaminated by species of *Fusarium*, through spores or mycelium trapped in irregularities on the seed surface. Therefore, seeds used in *Fusarium* assays should not be sterilized as these contaminants could potentially be eliminated. The assay is performed by plating 500 seeds (Figure 30) (20–25 seeds per petri dish) on Komada's selective medium (Komada 1975). This medium is selective for *Fusarium* spp. and is modified to have a pH 4.0 and no fungicide (benomyl) is added. The seeds are then incubated at 24/18°C with a 14/10 hour day/night photo period. Seeds are examined for *Fusarium* at 5 and 10 days after plating. Cultures are examined macroscopically for characteristic hyphae as well as pigmentation on the selective media. The presence of *Fusarium* is confirmed by microscopic examination (Figure 31). The sole appearance of **microconidia** is not diagnostic and demands further incubation of any cultures until banana- or canoe-shaped, **macroconidia** can be seen (Figure 32a). Identification of the fungus to the genus *Fusarium* is confirmed by macroconidia shape. Percent

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Ba	H	M	L
Bg	M	M	L
Bl	H	H	L
Cw	L	M	L
Fdc	L	H	L
Fdi	L	M	L
Hw	L	M	L
Lw	L	H	M
Plc	L	L	L
Pli	L	L	L
Pw	M	H	L
Py	L	H	L
Ss	H	M	H
Sx	M	M	H
Sxs	M	M	H
Yc	L	L	L

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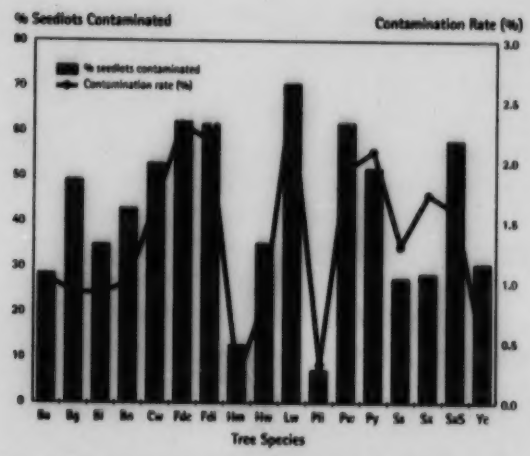

Figure 25 Proportion of tested seedlots showing evidence of being contaminated with *Fusarium* spp. and the mean contamination level (1992–2001).

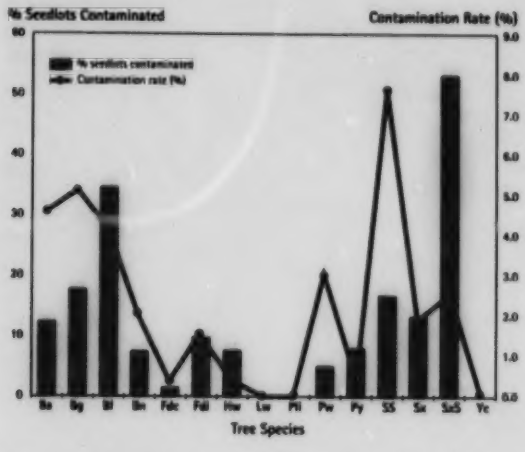

Figure 26 Proportion of tested seedlots showing evidence of being contaminated with *Coloscypha fulgens* and the mean contamination level (1992–2001).

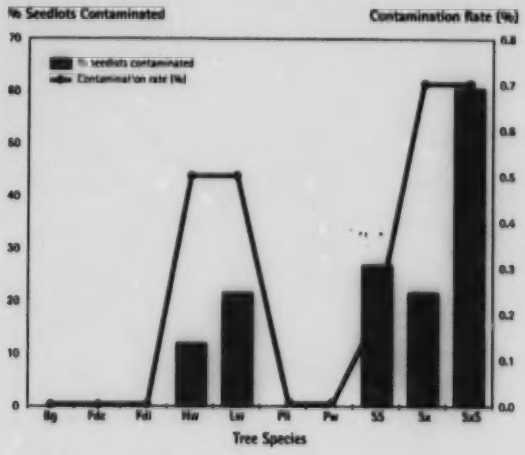

Figure 27 Proportion of tested seedlots showing evidence of being contaminated with *Sirococcus conigenus* and the mean contamination level (1992–2001).

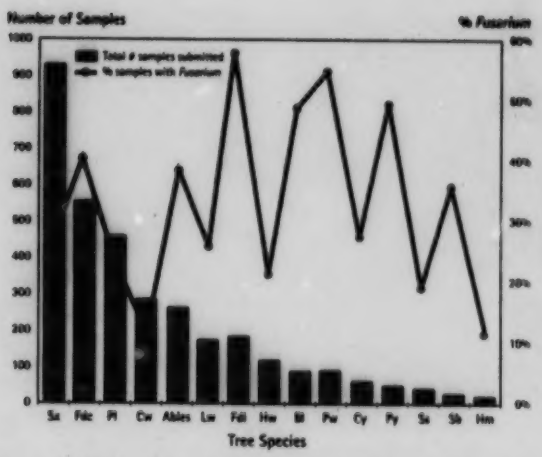

Figure 28 Total number of samples submitted and percentage of samples contaminated with *Fusarium* received at the Canadian Forest Service Health Clinic (1985–2001).

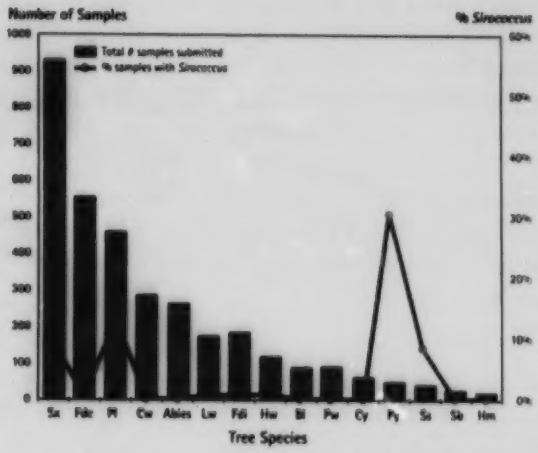

Figure 29 Total number of samples submitted and percentage of samples contaminated with *Sirococcus* received at the Canadian Forest Service Health Clinic (1985–2001).

Figure 30 Seeds are placed on culture medium in Petri plates under sterile conditions to determine the presence of potential seed-borne fungi.

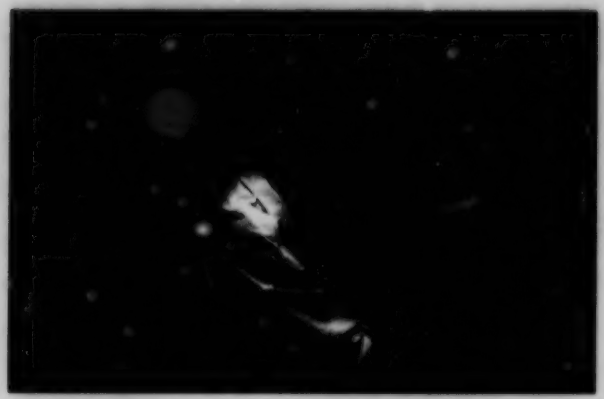

Figure 31 Positive identification of potential seed-borne fungi is made following microscopic examination.

contamination of seeds for the assayed seedlot is then calculated. Several different species of *Fusarium* can cause root rot of container seedlings with the major source of inocula being the seeds (Landis et al. 1990). Species of *Fusarium* are highly variable and can undergo morphological changes in culture. For species identification, all cultures must be grown from a single spore (Nelson et al. 1983). Identification is based primarily on macroconidial and conidiophore morphology and presence of absence of other spore types. Such identification can be time consuming and extremely costly; therefore we identify seed-borne *Fusarium* to the genus level only.

Caloscypha fulgens

Detection of *Caloscypha fulgens* within seeds requires the elimination of all surface contaminants on the seeds. These assays can be conducted by first soaking 250 seeds for 30 minutes in a glass container in a 30% solution of hydrogen peroxide (H_2O_2) at three times the volume of the seeds. The seeds should next be rinsed three times with sterile distilled water and surface dried on a paper towel. The seeds should be plated on 2% water agar and incubated at 15°C (light unimportant). Once every three days thereafter, the plates should be examined for blue or indigo-coloured, verrucose (warty) hyphae that branch at right angles (Figure 32b), characteristic of *Caloscypha fulgens*.

Sirococcus conigenus

Seed-borne *Sirococcus conigenus* also results in an infection and as for the cold fungus assay, it is necessary to surface sterilize the seeds. Similarly, 1500 seeds should be soaked for 30 minutes in a 30% solution of hydrogen peroxide (H_2O_2) at three times the volume of the seeds. The seeds should next be rinsed three times with sterile distilled water and surface dried on a paper towel. The seeds should be plated on 2% water agar and incubated at 20°C with 8 h light (900 lux) per day. Following this, the seeds should be examined for *S. conigenus* three days after plating and twice weekly for up to three weeks. Cultures are then checked for fruiting bodies (pycnidia) and two-celled, spindle-shaped spores (Figure 32c). *Sirococcus conigenus* is slow to form fruiting bodies. Therefore, it is important to transfer fungi that have not produced pycnidia or been identified as *S. conigenus* to separate plates so they do not contaminate other seeds. These are encouraged to fruit so they can be either identified or discounted not to be *S. conigenus*.

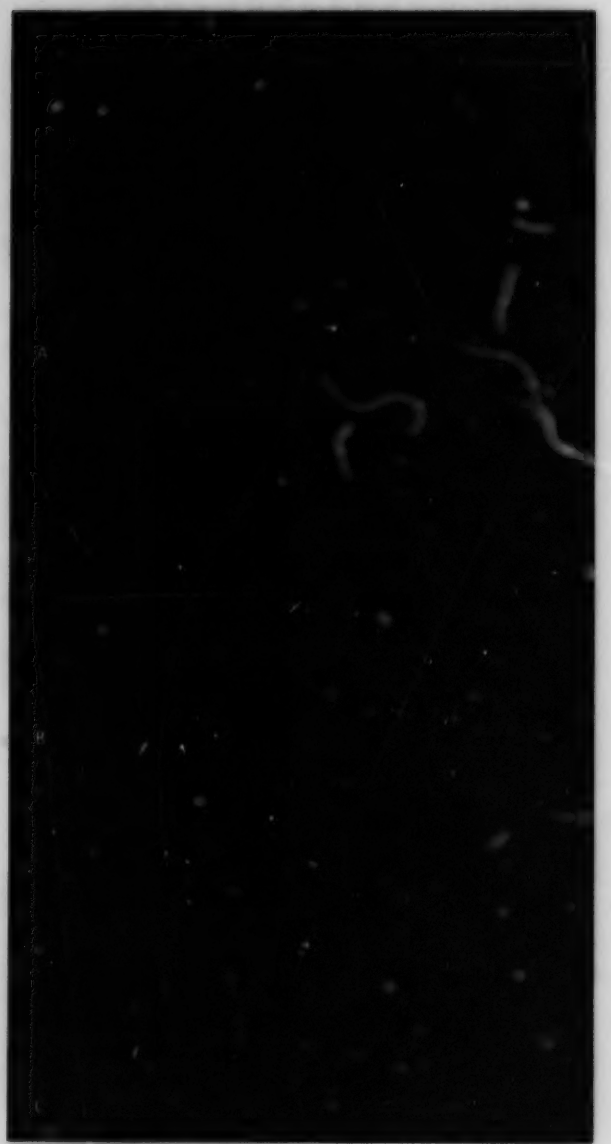

Figure 32 a) *Fusarium* macroconidia are characterized to be "canoe" or "banana-shaped." b) *Caloscypha* hyphae typically branch at right angles. c) *Sirococcus* spores are spindle shaped and two celled.

Interpretation of Assay Results

Testing for the presence of seed-borne pathogens provides useful information for nursery growers. As levels of contamination or infection within a seedlot rise, the potential to negatively affect seedling germination and growth becomes significant. Knowing the percentage of infected or contaminated seeds within any seedlot provides growers the option of taking steps to minimize their impact on seedling germination and growth. The level where the presence of seed-borne pathogens becomes significant is based on our understanding of each organism's life cycle as well as past records of its frequency of occurrence. Remember that thresholds for indicating significant levels of infected or contaminated seeds are guides only. Decisions regarding seed assay results must be tempered with other factors such as the economic value of specific seedlots and the unique environment (nursery) at which they are grown. While unlikely, very low levels of seed-borne contamination or infection do have a potential of spreading within a seedlot. However, potential risk to a seedlot increases as a threshold of infected or contaminated seeds is approached.

...thresholds for indicating significant levels of infected or contaminated seeds are guides only

Historical assay records indicate contamination or infection levels of 5% or greater within any seedlot to be significant for either *Fusarium* or *Caloscypha*. As seed-borne *S. conigenus* can become systemic in resulting germinants and spread via spores to infect adjacent seedlings, infection levels as low as 1% are significant.

When seed-borne *C. fulgens* and species of *Fusarium* begin to infect or contaminate seeds in a seedlot, steps should be taken to minimize the impact of these organisms (see "Seed Sanitation" chapter). The main strategies used for *Fusarium* and *Caloscypha* are aimed at minimizing the ability of each pathogen to spread within a seedlot. The main methods used to control the impact of *Sirococcus* infection in a seedlot are designed to eliminate the organism.

The results of fungal assays are available for each seedlot on the Seed Planning and Registry Information System (SPAR) under the seedlot test screen. If you are unfamiliar with SPAR and require access or training, contact the BC Ministry of Forests Tree Improvement Branch. For those familiar with SPAR, you can access seedlot tests by simply typing "v slt" on the command line and you will be prompted to enter a seedlot number. Otherwise, you can follow the menus from choice #1 = Seed/Cutting Lot Queries and then choice #2 = Seed Lot Query and, once a seedlot number is entered, press shift-F1 to get to seedlot tests.

The fungal assay results are also available in seedlot detail reports from SPAR. Fungal assay information is also forwarded on the sowing request label that is sent with each batch of seeds (Figure 33). This seedlot has been tested for *Caloscypha* (CAL) and *Fusarium* (FUS), but not for *Sirococcus*. This reflects the priorities indicated in Table 4. The level of *Caloscypha* is significant, but no *Fusarium* was found on the seeds from this seedlot.

Figure 33 A sowing request label illustrating presentation of fungal assay results.

...contamination levels of 5% or greater within any seedlot may be significant for either Fusarium *or* Caloscypha. S. conigenus *can infect adjacent seedlings and infection levels as low as 1% are significant*

Cone collection and post-collection handling of cones is very important in determining seedlot quality. Information specific to cone collection and handling can be found in *A Guide to Collecting Cones of British Columbia Conifers* (Eremko et al. 1989); *A Field Guide to Collecting Cones of British Columbia Conifers* (Portlock 1996), or chapter 15 in *Regenerating British Columbia's Forests* (Leadem et al. 1990). Several aspects are worth emphasizing here. The first step is locating stands that meet your demands (e.g., Amabilis fir in the Maritime seed planning zone above 800 m) followed by monitoring the crop throughout cone development. The importance of pre-collection evaluations cannot be overstated. One should have a very good idea about cone and seed maturity, potential yield and degree of pest activity before collecting cones. Sampling should become more frequent as cones and seeds are approaching full maturity (generally August to September).

Collection Methods that Minimize Seed-borne Disease and Other Problems

Seed orchard cones are generally not affected by *Caloscypha* or *Sirococcus*, but are susceptible to contamination by *Fusarium* spp. Cones collected from natural stands are subject to *Caloscypha* and *Sirococcus* infection as well as contamination by *Fusarium* spp. Often cones are observed to be covered with moulds other than these and in many cases fungi present on cones are not pathogenic in nature. These can lead to other indirect losses however such as those caused by casehardening. In seed orchards cone contamination by *Fusarium* spp. likely occurs via spores, prior to collection. These contaminations are beyond the control of cone collectors. However, more control is available when collecting in seed orchards compared to natural stands and three things can be done to prevent further contamination and additional spread of the fungus.

One should have a very good idea about cone and seed maturity, potential yield and degree of pest activity before collecting cones

First, if possible, collect cones during dry weather. Second, store cones in new sacks or steam or hot water-sterilize old sacks before use to prevent contamination from a previous year's collections. Finally, store filled sacks following the general recommendations for all species described in Portlock (1996) to ensure the best conditions for drying while minimizing their exposure to conditions that favour the spread of fungus. These recommendations include the following:

Cones from natural stands may be collected using any of several methods, including climbing, felling, and collecting from helicopters and squirrel caches

- Fill cone sacks only one-third to one-half full to avoid heat buildup
- Ensure that filled sacks are tied at the top to allow for cone expansion
- Store filled cone sacks on their sides, not upright, in a dry location off the ground
- Change sacks if they become wet during cone picking
- Store filled sacks in the shade during picking
- Move sacks daily from the collection site to interim storage
- Turn sacks when and if required (dependent on cone moisture content)
- Cone and seed evaluations and inspections should continue during interim storage
- Consult references for species-specific recommendations.

Seed orchard cones are usually picked by hand and post-collection contamination is minimized. Cones from natural stands may be collected using any of several methods, including climbing, felling, collecting from helicopters and squirrel caches. Each method exposes cones to contamination and infection and steps can be taken by pickers to minimize the impact of seed-borne disease in each case. As in seed orchards, cones should always be stored in either new or heat-sterilized dry sacks (use most cost efficient) to reduce the risk of *Fusarium* contamination.

Cone collection method and efficiency will vary with species and crop intensity. Some natural stand collections are made by climbing trees when cones are mature and either placing them directly into containers or more often, by shaking branches to knock cones to the ground. Often, it is expedient to climb many trees one after another, knocking large numbers of cones to the ground, returning to collect these

later. If all the cones knocked to the ground can not be collected immediately they risk exposure to *Caloscypha*, especially if left for extended periods in cool wet weather. Cones collected in this manner should be removed from the ground as quickly as possible. Otherwise, spread tarpaulins under the trees to separate the fallen cones from the duff. A common *Sirococcus* point of entry to the seed handling system occurs when old cones which have fruiting bodies on them (Figure 34), are included with the new cones collected from under trees. Ensure that pickers are diligent to avoid the inclusion of old cones in collections.

Figure 34 Avoid collecting old cones with *Sirococcus* fruiting bodies (pycnidia) on them.

Coordinating cone collections with logging is an efficient method of obtaining seeds. These situations offer opportunities to harvest cones from felled trees when falling operations and cone maturity coincide. Similar precautions to those used when knocking cones from standing trees should be employed when collecting from logging slash. Cones should be collected as quickly as possible after trees have been cut to avoid heating and to minimize their contact with forest duff and any *Caloscypha* inoculum. Similarly, steps should be taken to avoid including old cones and the potential introduction of *Sirococcus* into the collection.

Aerial collections using a helicopter allow cones to be harvested from the tops of trees (Figure 35a). Trees with heavy cone crops can easily be selected from the helicopter and with appropriate planning and coordination this can be a cost-effective method of collecting cones providing a broader range of choice through the opening of otherwise inaccessible areas. This is especially well suited to *Abies* spp. and others in which the cones are concentrated at the top of the crown. Helicopter collection allows little opportunity to assess phenotype, although it is generally accepted that helicopter collections offer a degree of disease avoidance, similar to collections from seed orchards. However, helicopter time is expensive and without precautions, the introduction of *Caloscypha* or *Sirococcus* can negate an otherwise high quality collection.

In order to optimize collection timing, monitor potential cone crops and choose helicopter cone collection areas before cones reach maturity. When collections are made, cone-bearing branches are either cut from treetops or cones are raked into a basket. With both methods, cone-bearing branches are dropped into a central area in the forest stand (Figure 35b). To minimize cost, cones are usually collected this way for several hours resulting in large piles of cones and branches to be sorted later. Spread tarpaulins on the ground, before the helicopter begins work, to minimize contact between cones and the forest duff. During hot weather, considerable heat can build-up within these piles. To guard against this, workers should spread the pile. In some situations, cones may not be separated from the branches and twigs for one or two days. This constitutes a risk to *Caloscypha* infection especially during wet weather. Western redcedar and western hemlock stand a risk of germinating under these conditions (Figure 36). During wet weather or those with heavy night dew, avoid leaving cone piles on the ground overnight. Take care also when sorting the cone/debris piles to avoid including old cones that might be infected with *Sirococcus*.

Figure 35 a) Helicopter cone collection from the top of a tree using a cone rake. b) Once cones are collected using the cone rake, they are collected in piles on the ground where they are potentially exposed to *Caloscypha*.

Figure 36 Premature germination of western redcedar in the cone.

The BC Ministry of Forests has not encouraged cone collection from squirrel caches since the late 1970s. It is briefly mentioned here to reinforce what we know with regard to the seeds or cold fungus. Cones have been traditionally collected from squirrel caches because it is relatively inexpensive and the collection period is much greater than with other methods. Observations appear to indicate that squirrels do not begin significant storage of cones until the seeds are mature. However, it must not be assumed that all cones and seeds are mature. Cached cones may require extra care and handling in interim storage. After dropping cones from trees, squirrels gather and place cones in piles or caches. These caches are usually located in cool shady areas and their biggest threat from seed-borne fungal pathogens comes from *Caloscypha*. If cones must be collected from squirrel caches, follow these recommendations to improve seed extractability and reduce the risk to seed-borne infection. Only collect cones from the top of the cache—buried, wet, or excessively dirty cones must be left behind. Clean collected cones of as much debris as possible prior to storage and give close attention to proper drying, as they are often wetter than cones collected using other methods.

Cone Handling and Seed Quality

Cone handling during and following collection can dramatically influence final seed quality as well as the incidence of seed-borne disease. Here we focus on the impacts that post-collection cone handling (interim storage) can have on seed-borne disease as well as overall seed quality. Options for impacting insect damage are limited as most feeding has already occurred. The exception is coneworm caterpillars that continue to cause damage until they pupate. Cone and seed processing may need to proceed as quickly as possible depending on population and feeding levels. In the interim cones should be kept cool to minimize caterpillar activity and further damage.

Understanding the lifecycles of the three major conifer seed diseases reveals that each of them can become seed-borne while seeds are still in the cones. Initial contamination or infection may take place while cones are still on trees. This applies most often to contamination of cones by *Fusarium* spp., although to a lesser extent *Sirococcus* infection is technically feasible in this manner as well. *Caloscypha* infects cones when they contact the forest duff. Once cones become contaminated or infected, spread of the fungus is encouraged by moist and cool conditions. These conditions should be avoided during the period that cones are being transported or stored prior to seed extraction.

Freshly picked cones are very moist and this moisture must be removed gradually to mimic the natural maturation process and prevent over-heating and/or casehardening of the cones

Freshly picked cones are very moist and this moisture must be removed gradually to mimic the natural maturation process and prevent overheating and/or casehardening of the cones. Collecting immature cones or picking them during wet weather will compound moisture problems. For moist cones reduce the volume per sack to promote uniform drying.

Store cones in sacks under shelters (Figure 37) exposing them to freely circulating cool air to gradually remove moisture. The weave of the cone sack should not allow released seed to be lost. It is important that sacks are not stored in direct sunlight as overheating can damage cones, but they also should not be allowed to remain wet for excessively long periods as this will encourage the spread of any fungal growth. Remember that air movement is more beneficial to drying than light or heat and that shade or

Figure 37 Cones should be stored on well-ventilated racks in clean dry sacks.

indirect light with good airflow is essential. During pre-conditioning one can sometimes see a mass exodus of some insects exiting from the cone sacks as they begin the overwintering phase of their lifecycle. These insects will generally cause no further damage, but they can allow populations to build up in adjacent areas and one should consider eradication measures to avoid this.

Cone Transport

Transportation of cones from interim storage to the extractory is an important aspect of post-collection handling as seed quality can be degraded by improper transport. The keys to proper transport is to provide good circulation around the cone sacks, maintain a cool temperature and limit the time in the transport vehicle. Proper circulation can be accomplished by using pallets to separate cone sacks. For most species cone sacks should be two deep followed by another pallet. For serotinous lodgepole pine it is acceptable to stack cones sacks to a depth of four to six sacks separated by a pallet.

The preferred transport method is in a refrigerated, closed van or trailer maintained at a temperature between 5 and 15°C. If cones are quite moist because of reduced or no interim storage (western redcedar and western hemlock) the vents must be open to allow for the removal of moist air. Non-refrigerated vehicles may be used to transport serotinuous lodgepole pine or collections that have been well conditioned. Transport time should be minimized with a suggested maximum transit time of 24 hours.

The keys to proper transport is to provide good circulation around the cone sacks, maintain a cool temperature and limit the time in the transport vehicle

This chapter has been divided into four sections: cone storage and handling, cone processing (kilning and extraction), seed processing, and seed upgrading. The first three are considered part of the standard cone and seed processing steps applied to each seedlot and illustrated in Figure 38–although all steps may not be performed on every species or seedlot. The appearance of processing materials at various representative stages are depicted in Figure 39. Seed upgrading may be performed at a later date to improve seedlot characteristics.

Cone Storage and Handling

Once cones arrive at the processing facility, promptly unload the cone sacks from the transport vehicle and place them onto a racking system (similar to interim storage) until cone processing begins. As in interim storage, protect the cone sacks from the elements, and allow ventilation for further drying (curing) of the mature cones (Figure 40). Turn cone sacks to facilitate uniform drying of the cones. The frequency of turning sacks is dependent on cone moisture content. If cones are still quite moist when received it is beneficial to turn the sacks daily, reduce the cone volume in each sack, or spread the cones on trays. Serotinous lodgepole pine is an exception and the cone sacks can be stacked without adversely affecting seed quality (Figure 41). Caron et al. (1993) found that white spruce can benefit from cone storage as much as from stratification in terms of germination capacity. In Douglas-fir, Sorensen (1991) showed that low humidity, high temperature cone drying conditions decreased the germination capacity and rate, but that prolonged stratification corrected this reduction. Proper cone storage can have a significant effect on seed quality.

During cone curing, moisture content is visually monitored (via cone opening) and a random sample of cones should be obtained to perform a cone and seed evaluation as soon as possible after receipt (Figure 42). This will provide information on: cone and seed maturity, presence and extent of fungi, presence and damage caused by insects, and other indicators that will allow staff to prescribe and prioritize subsequent cone handling and extraction activities. An example of a cone and seed evaluation form is presented in Appendix 3.

The *Abies* spp. and other seedlots with high moisture levels may have their cones placed on open, stackable trays in cool (10–15°C) conditions with additional fans for ventilation to facilitate the drying of cones (Figure 43). The *Abies* spp. are treated differently as cones disintegrate naturally compared to the cone opening of all other species. The handling practices for cones collected immaturely has been referred to as after-ripening or artificial ripening. This was first suggested by Silen (1958) to expand the window for cone collection in Douglas-fir and was subsequently studied in a variety of species. A thorough review of seed maturity including the artificial ripening of seeds was provided by Edwards (1980). Today, less consideration is given to artificial ripening, except in *Abies* spp., as it is generally accepted that maximum seed quality occurs at the point of natural seed release. A much larger proportion of seeds is also produced from seed orchards today allowing collections to be performed on an individual tree basis at the peak of seed maturation.

Prior to kilning, cones may be cleaned on a vibratory screening machine to remove debris and seeds that have been released from the cones (Figure 44). The removed material must then be cleaned to separate the debris from the seeds. Next, dry these seeds to the moisture content of kilned seeds (7 to 8%). The kilned and unkilned seeds can then be combined for further processing. In some seedlots there will be no seeds released prior to kilning, but for other seedlots in which cones have begun flexing, the number of seeds released may be substantial. Cone processing includes cone conditioning, cone cleaning, kilning, and extraction of viable seeds.

Cone processing includes cone conditioning, cone cleaning, kilning, and extraction or viable seeds

Figure 39 The appearance of processing materials a) unkilned cones, b) kilned cones, c) winged seeds, and d) dewinged seeds of interior spruce (Sx).

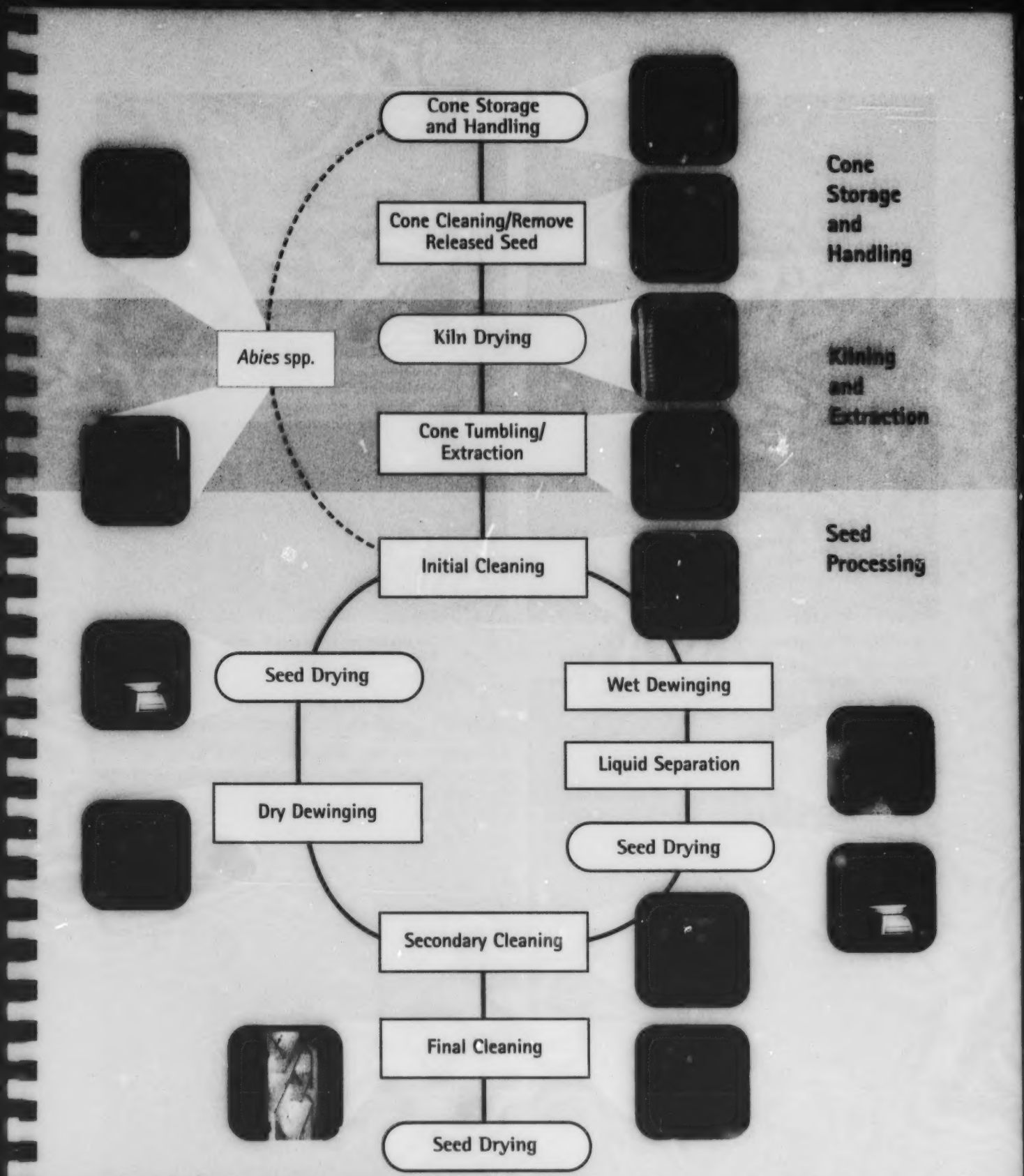
Cone Storage and Handling
Cone Cleaning/Remove Released Seed
Kiln Drying
Cone Tumbling/ Extraction
Initial Cleaning
Seed Drying
Dry Dewinging
Wet Dewinging
Liquid Separation
Seed Drying
Secondary Cleaning
Final Cleaning
Seed Drying
Abies spp.
Cone Storage and Handling
Kilning and Extraction
Seed Processing

Figure 38

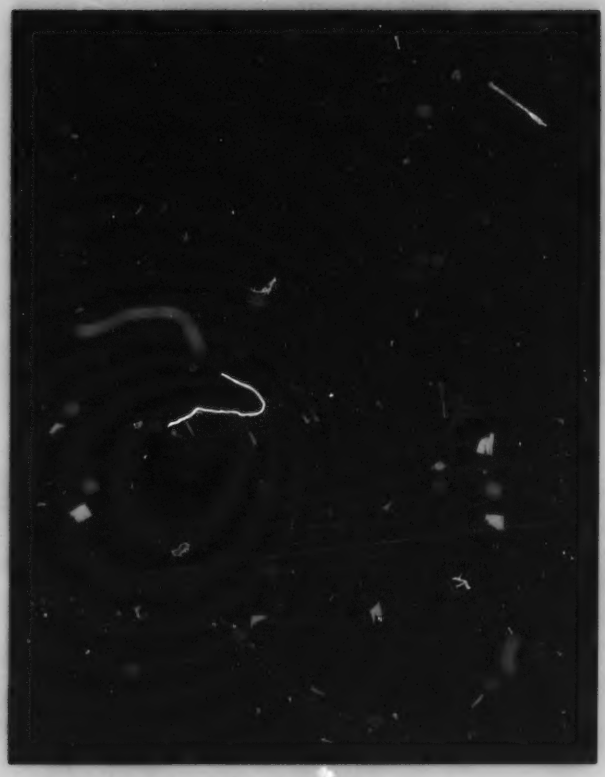

Figure 40 A typical cone sack racking system.

Figure 41 Stacking of cone sacks for serotinous lodgepole pine.

Figure 42 Materials used in a cone and seed evaluation.

Figure 43 Conditioning cones of *Abies* spp. in stackable trays with additional ventilation provided by fans.

Figure 44 Two types of equipment used to remove released seeds and debris from cones, before kilning.

Kilning and Extraction

Kilning refers to drying cones in a controlled, warm, dry environment to flex the cone scales and allow seeds to be extracted. If kilning is applied to very moist cones the outer portion of the cone scale may lose moisture and partially flex causing the scale to set in a semi-open position preventing seed release (Edwards 1985). This condition is called case-hardening. This term is also used to describe cones that will not open due to fungi, insects, excessive pitch covering the cones, and overheating during interim storage or transport.

There are two main types of kilns used to remove moisture and induce flexing in conifers: rotary and batch (Figure 45). The essential elements in any kiln design are (a) heat for evaporating moisture, (b) air circulation to conduct heat, (c) control of temperature and humidity to prevent injury to the seeds (Rietz 1941), and (d) a tray or shelf system to expose cones to the air current in batch-style kilns. A rotary kiln is a temperature controlled chamber that rotates cones in a large wire drum allowing released seeds to fall through the drum and exit the kiln environment. The rotation speed and duration of the kiln can be adjusted. Each rotary kiln can process only one seedlot. The batch type kiln, similar to lumber kilns, introduces cones on dollies and exposes the

cones to controlled heat and humidity for a specific duration. Rotary kilns offer the advantage of automatically removing released seeds from the hot, dry environment (combining kilning and tumbling), but batch type kilns allow greater flexibility in the number and size of seedlots that can be treated at one time. An initial investigation of the effect of kiln temperatures on germination indicated that germination was not reduced by the range of kilning temperatures used in seven BC conifer species (Rappaport 1996).

Kilning refers to drying cones in a controlled, warm, dry environment to flex the cone scales and allow seeds to be extracted

The duration and settings for temperature and humidity form the basis of the batch kilning program. In general temperature is ramped up to one of three peak temperatures: 30°C for the low dormancy species (western redcedar and western hemlock), 60°C for serotinous lodgepole pine and black spruce, and 40°C for all other coniferous species that are kilned. The kiln usually runs on a 17 hour timeline overnight with temperature ramping up gradually, over a two to five hour period, to the peak temperature. This temperature will be maintained for several hours and then ramped down in the morning to the ambient temperature. Relative humidity in the kiln will initially be high, corresponding to the relatively high cone moisture content, and will then decrease over the kilning period as cones lose moisture. The kiln control parameters are important as low initial relative humidity, high initial temperatures or a sudden change in cone moisture content can cause casehardening of the cones.

The loading and unloading of the kilns can differ between facilities. In some, the loading of kilns will still be performed manually, while mechanization has been incorporated into others (Figure 46). The most important consideration is to avoid contamination of materials between seedlots; a thorough cleaning of all equipment must occur between seedlots (i.e., by vacuum). This applies to kiln loading and unloading, and to all other phases of cone and seed processing. If cone opening has occurred in the sacks then one should ensure all released seeds are also incorporated into the processing cycle (i.e., turn cone sacks inside out to check for released seeds). If burlap sacks are turned inside out before filling, the inner seam will probably not "catch" seeds and efficiency of extraction can be improved. Not all burlap is the same (e.g., weave differences) and sack construction can also vary—be aware.

...to avoid contamination clean equipment thoroughly between seedlots

Following kilning in a batch-style kiln, the seeds are extracted from the cones in a large mesh cylinder referred to as a tumbler (Figure 47). The speed of rotation and angle of the cylinder are usually adjustable to allow for optimization by species. Seeds will fall through the mesh screen and onto a conveyor belt that will collect seeds and debris in a plastic bag. Spent cones

Figure 45 The two main types of kilns used for opening cones a) batch-type and b) rotary.

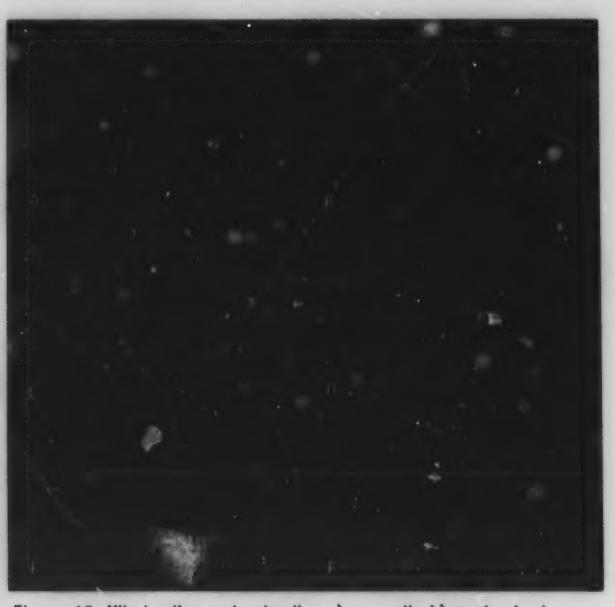

Figure 46 Kiln loading and unloading a) manually, b) mechanized stacking of batch-style kiln trays, c) mechanized destacking of batch-style kiln trays in preparation for tumbling, d) cleaning of equipment between seedlots, e) loading of rotary kiln, and f) collection of seeds outside rotary kiln.

traverse the length of the mesh cylinder and for small-coned species they are vacuumed out of the processing plant to an outside holding area. For large-coned species, cones are manually removed from the extraction area.

Extraction is a critical point in processing. If all viable seeds are not removed from the cones at this point in time it can have a large impact on yield. If it is determined that sufficient viable seeds still remain in the cones and are extractable, the seedlot, or a portion of it, may be rekilned to improve cone opening and extraction efficiency. It is important to determine through cutting tests whether unextracted seeds are viable, as many 'empty' seeds are routinely retained in the cone. Excessive tumbling should also be avoided as it introduces additional debris to the seedlot, reducing processing efficiency and possibly damaging seeds.

Seed Processing

Following the removal of seeds from the cones, seed processing is initiated. Seed processing deals with the purification of seeds, reduction in moisture content, and removal of non-viable seeds. Initial cleaning is the first step in seed processing and is primarily concerned with the removal of debris from a seedlot that may damage the seeds or impede processing. A 'scalper' or multi-screened vibrational seed cleaner uses metal screens of varying opening size, shape, and arrangement to separate seeds from debris (Figure 48). Examples of debris are cone scales, foliage, pitch, rocks, and other inert matter (Figure 49). Choice and order of screens as well as vibrational speed are based on the species and type of debris in each seedlot and are important decisions for efficient and successful seed cleaning. The stickiness of seeds can also be problematic for seed processing or nursery sowing. Minimize stickiness by placing the seeds in a cool environment prior to use or use scientific grade talc or a seed flow lubricant during processing or sowing.

Seed processing deals with the purification of seeds, reduction in moisture content, and removal of non-viable seeds

The seeds separated during initial cleaning will then be dewinged to remove the seed wing from its attachment to the seed coat. Dewinging generally occurs in a rotary drum or cement mixer in which rotation speed and angle can be controlled and water can be added if required (Figure 50). The seed wings are blown off in the dewinger or separated later in the aspirator or on the gravity table. The dewinging stage has prompted the development of various pieces of

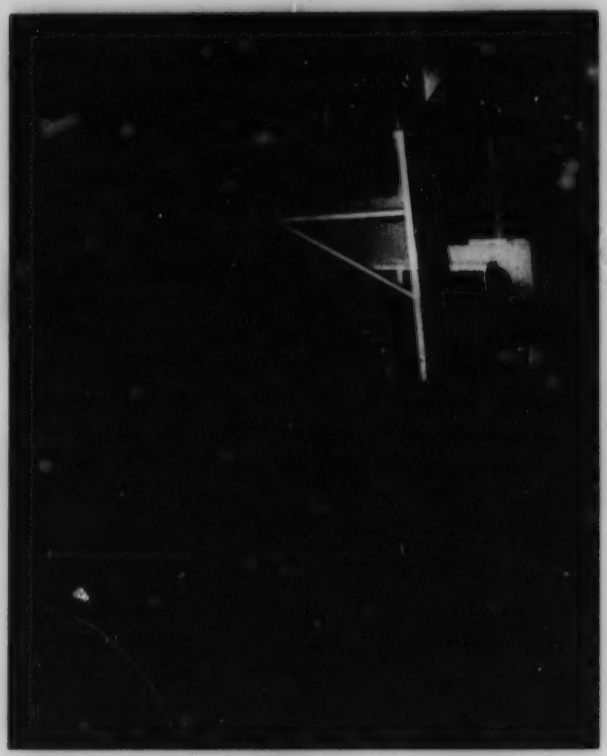

Figure 47 a) A cone tumbler and b) a close-up of the wire mesh screen used to separate seeds from cones.

Figure 48 a) Initial cleaning performed on the scalper and b) a close-up of the seeds running over the screens.

Figure 49 Examples of debris commonly found in seedlots before processing.

equipment including augers and brush dewingers. For small seedlots dewinging is often accomplished by hand. For species of spruce and pine, wet dewinging is employed as the addition of moisture causes the wings of these species to enlarge and cleanly detach from their connection with the seed coat. Species which are wet dewinged also subsequently undergo a very brief water bath which helps to separate particles denser than water (i.e., rocks and pitch) which sink to the bottom of the liquid separation tank (Figure 51). Prior to further processing the wet dewinged seeds are dried to a storage moisture content target of approximately 8%. For the other Pinaceae species dewinging is performed 'dry' (most efficient at seed moisture contents less than 15%) and wing removal results from mechanical friction and breaking the connection of the wing and the seed coat. In some species, drying of seeds prior to dewinging results in the wing becoming more brittle and breaking from the seeds more easily. Seeds from species in the Cupressaceae (western redcedar and yellow-cedar) are not dewinged. Wet dewinging results in much 'cleaner' looking seeds that will not release more debris (wing material) over time, but not all species respond to 'wet' dewinging. Improvements to dry dewinging have included the use of foam inserts or other foreign objects into the drum to increase frictional forces and improve dewinging efficiency. Dewinging is a stage in which the probability of seed damage is higher and it is important that the activity be as brief as possible to accomplish the required product. In particular, species with resin vesicles can be damaged and require additional care during all stages of cone and seed processing. Secondary cleaning may or may not be performed depending on the purity of the seedlot after dewinging. It may be performed on the scalper, sizer (basically a smaller-sized scalper), or on a fanning mill which includes aspiration in an air column with scalping. Secondary cleaning reduces debris volume in preparation for final cleaning and

Minimize stickiness by placing the seeds in a cool environment prior to use or use scientific grade talc or a seed flow lubricant

Figure 50 a) Dewinging performed dry in the large batch machine and b) wet dewinging being performed in a cement mixer.

Figure 51 Two different pieces of equipment used for liquid separation.

can be used to size seedlots. Seed sizing has been a subject of great debate as conflicting results have been found concerning its benefits. In the southeastern United States, seed sizing is performed on loblolly pine and the increasing uniformity of germination speed may justify the sizing practice (Dunlap and Barnett 1983, Barnett 1989). Sizing also appears practical in the southeastern US as quantities of seed per size class (or family) may reach 20 kg or more. For BC species that have been studied there does not appear to be justification for seed sizing and the small request sizes often faced do not improve the feasibility of this practice. Sizing may sometimes be performed to improve final cleaning

Dewinging is a stage in which the probability of seed damage is higher and it is important that the activity be as brief as possible to accomplish the required product

separation efficiencies. For Sitka spruce it was determined that although seed size differences between parent trees are significant they were minor compared to the effect of pretreatment, having little importance operationally (Chaisurisri et al. 1992). Douglas-fir seed size showed significant differences between families, but correlations between seed size and germination capacity and rate were weak (St. Clair and Adams 1991).

In a study looking at sorting seven interior spruce families into four fractions, the smallest seeds (passing through a 1.37 mm holed screen) had the lowest germination capacity and rate (Perkins 1998) and removal of these seeds which occurred in all families would have little impact on genetic variability, but may significantly improve seed quality. The decision to size seeds is complex and will depend upon species attributes, quantity of seeds, nursery seeder used, and finally, as the bottom line, the cost effectiveness of sowing sized request fractions which can vary greatly by nursery.

Cutting tests are used to calibrate settings and determine if acceptable separations are occurring

Final cleaning is the final removal of debris particles, which should have been minimized through previous processing, and the removal of empty, immature, and non-viable seeds. Two pieces of equipment can be used for final cleaning: aspirators or the gravity table. The aspirator or pneumatic separator uses an adjustable air column to separate seeds based on terminal velocity which is influenced by specific gravity, size, shape, and surface texture (Edwards 1979). Aspirators may have several, usually three, outlets for seed discharge. These are commonly referred to as light, mid, and heavy seed fractions. Cutting tests are used to calibrate air flow settings and determine if acceptable separations are occurring. The machine is set up to separate the heavy seeds considered filled and viable from the light fraction consisting of empty seeds and debris. The mid fraction is usually a combination and commonly has to be re-run, with adjusted settings, to separate out the viable seed. Various configurations on this central concept have been constructed (Figure 52) and the 'aspirator' is a common piece of equipment found in most seed processing facilities. A review of air separation and a detailed description of one configuration of a laboratory aspirator is presented in Edwards (1979).

The gravity table, originally used in the mining industry, is the primary tool used for final cleaning at the BC Ministry of Forests Tree Seed Centre (Figure 53). Seeds are separated across an inclined deck that moves in two directions—up and down, and backwards and forwards. An air current is also present from below the deck. Although it requires a great deal of dedication and seed knowledge on the part of the technician it can produce excellent separations. The gravity table is initially overwhelming as the operator has many variables to control.

Figure 52 Various configurations of aspirators used for final cleaning.

The air current blown through the gravity table deck is strong enough to lift the light seeds slightly off the surface. These

Figure 53 The gravity table used for final cleaning.

light seeds, not in contact with the deck will run to the lower end of the deck due to the force of gravity. The heavier seeds, in contact with the deck, will be moved upwards with the reciprocating (two-dimensional) motion of the deck. The outcome of light seeds running down the deck and heavier seeds running up the deck initially seems counter-intuitive until one recognizes that different forces are used to move these fractions in their respective directions. Separations are performed on the gravity table by placing dividers on the discharge end of the deck separating the seeds into heavy, mid and light fractions similar to the aspirators. The placement of dividers is determined through cutting tests and it is common that more than one 'run' is required for each seedlot or fraction thereof. Separate runs on the gravity table usually include changes to settings and adjustment of dividers based on additional cutting tests.

...dead filled seeds and viable seeds will both imbibe moisture in a similar manner, but upon drying the dead filled seeds will lose moisture much more rapidly and allow for a separation to occur

Following final cleaning the seedlot is blended to ensure a homogenous product. A seedlot must meet the registration requirements for purity (97%+) and moisture content (4.9 to 9.9%) before the seedlot is placed into long-term storage at -18°C. After final cleaning it is common for some species (e.g., *Abies* spp. which have not undergone any kiln drying) to require further drying before storage. Once registration requirements of moisture content and purity have been met the seedlot will be sampled for seedlot tests: germination, seed weight, x-ray, and possibly fungal assays.

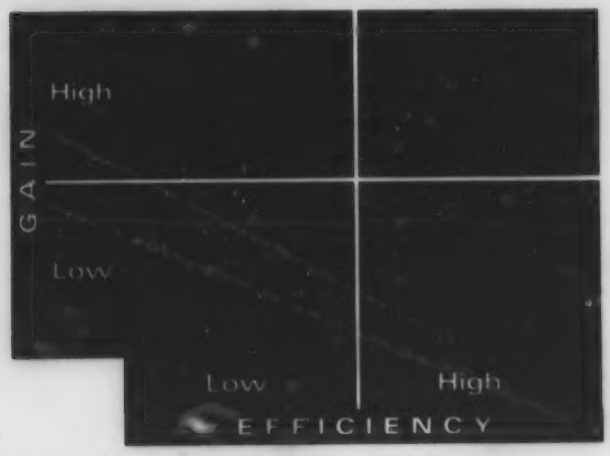

Figure 54 Tradeoffs between upgrading gain and efficiency.

Seed Upgrading

Seed upgrading refers to a variety of methods used to improve the quality of a seedlot. Seed upgrading often refers only to improvements in germination capacity, but upgrading or enhancing seed quality will be used here to also describe improvements in seed characteristic such as purity, moisture content, and possibly rate or uniformity of germination. In upgrading, and to some extent in conventional seed processing, one is dealing with a tradeoff between the gain in a particular trait (i.e., germination capacity) and the efficiency (i.e., yield, which is the amount or proportion of viable seeds retained) of the separation as illustrated in Figure 54. The optimum situation occurs when we achieve a high gain and a high rate of treatment efficiency. This occurs when a discrete difference exists in some characteristic, such as density, between the seeds we want to retain and remove. If gain or efficiency is reduced, the decision to upgrade will be an economic one due to the cost per increment of gain. Another important aspect is initial seed quality. If initial quality is high there will be little difference between the product before or after upgrading. It is generally recognized to be easier to improve a lower quality seedlot (e.g., from 75% to 85%) than to take a high quality seedlot from 92% to 96%. There appears to be diminishing returns to seed upgrading if initial seed quality is already high.

Separation of seedlots into discrete categories requires the identification of clear parameters. When dealing with immature seedlots possessing a great deal of variability, upgrading becomes difficult or impossible within reasonable efficiency limits. A procedure that has gained a great deal of international attention with conifers is the incubate-dry-separate (IDS) method. While many other methods rely on the physical characteristics of seeds this procedure separates seeds based on physiological principles. The basic principle is that dead filled seeds and viable seeds will both imbibe moisture in a similar manner, but upon drying the dead filled seeds will lose moisture much more rapidly and allow for a separation to occur. The live seeds actively bind moisture and more energy is required to remove this moisture compared to dead filled seeds. A thorough review of the procedure can be found in Bergsten (1993). Several research papers have been published illustrating how this method can improve seedlot quality (Simak 1984; Bergsten 1988; Downie and Bergsten 1991; Downie and Wang 1992; Poulsen 1995; Vanangamudi et al. 1993).

In BC, the IDS and related techniques have received a great deal of research and attention for improving seedlot quality (Edwards and Banerjee 1989; Banerjee and Scagel 1992; Kolotelo 1993). Results have generally been good when trying

to improve poor quality seedlots, but many seed owners are looking to IDS, or related technologies, to raise germination capacity to accommodate single-seed sowing (≥95% germination capacity). Although the technology can provide this level of improvement, not all seedlots can be upgraded to this level efficiently. One may have a great deal of flexibility in efficiency if the goal is single-seed sowing as one can afford to throw away approximately half the seeds by reducing the sowing factor from two to one seed per cavity.

Although no nurseries in BC currently use the IDS technology, there are nurseries which engage in upgrading prior to sowing. Many nurseries simply soak the stratified seeds in a water bath and skim off the floating material consisting of debris and seeds which are lighter than water when imbibed (Figure 55). Success with this technique is mainly a matter of luck in having undesired seeds and debris float while the viable seeds sink. It is a quick and easy method that will not work for all species or all seedlots within a species. The technique will ensure that all the seeds are evenly and adequately hydrated prior to sowing and this will help the crop germinate rapidly and uniformly.

Figure 55 Separation of debris and empty seeds through the soaking of stratified seeds.

Another method, the PRE-VAC technique, is used to remove seeds that have seed coat damage (Downie 1999). The procedure works by placing dry seeds in a water-filled chamber, creating a vacuum to evacuate air from cracks in the seeds and then releasing the vacuum. The water will more easily enter the mechanically damaged seeds causing them to lose buoyancy and sink. This allows for a separation to occur. The method is specific to mechanically damaged seeds and although uncommon using conventional processing today, it is a viable method of improving past seedlot errors.

Tests are performed on seedlots to quantify their germinability, purity, size, and moisture content. For additional details on the methods and procedures for testing tree seeds in Canada the reader should consult Edwards (1987). A key aspect of good seed testing is proper sampling. The test result for a seedlot is only as good as the sample taken to represent the seedlot. Keywords for sampling are random and representative. Guidelines for sampling, which account for seedlot size, are provided by the International Seed Testing Association (ISTA 1999). Sample sizes used in each of the four tests described apply regardless of seedlot size (see Figure 56).

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A seedlot is required to pass two tests for registration and reforestation use on Crown land in BC—purity and moisture content. The purity of a seedlot is the weight of pure seeds divided by the weight of pure seeds plus debris and is presented on a percentage basis. Debris commonly found in seedlots includes cone fragments, pitch, needles, seed wing remnants, pollen cones, insects, damaged seed, rocks, and other inert matter. The purity of a seedlot must be 97% or better for Crown land reforestation in BC. Exceptions to this rule may occur if it is thought that further processing will adversely affect seedlot quality.

$$\text{Purity (\%)} = \frac{\text{pure seed weight}}{\text{pure seed weight + debris weight}} \times 100$$

The moisture content of seeds is calculated on a fresh weight basis[3] as the fresh weight minus the oven dry weight (i.e., weight of water). This result is divided by the fresh weight. Moisture content is commonly expressed on a percentage basis by multiplying this result by 100. The ovendry weight is the weight of seeds with 0% moisture and is obtained by placing the seeds in an oven at 103°C for 17±1 hours and weighing the resulting seeds. Seeds must have moisture content between 4.9 and 9.9% to be registered and stored for use for Crown land reforestation in BC.

$$\text{Moisture content (\%)} = \frac{\text{fresh weight − ovendry weight}}{\text{fresh weight}} \times 100$$

Figure 56 The sample sizes used for seed testing a) purity, b) moisture content, c) seed weight, and d) germination.

Seed size is a general term used to describe the relative size of tree seeds. The weight of 100 or 1000 seeds is the test used to quantify the weight of tree seeds. For BC species the weight of 100 seeds is based on eight 100-seed replicates and varies from 0.13 g for western redcedar to 5.26 g for ponderosa pine. The term seeds per gram (SPG) is the test result that appears on the seed planning and registry (SPAR) system and is used in sowing calculations. The SPG value is a function of seed weight and the purity of a seedlot relative to its storage moisture content.

$$\text{Seeds per gram} = \frac{100}{\text{weight per 100}} \times \frac{\text{purity (\%)}}{100}$$

The SPG varies from an average of 19 for ponderosa pine to 792 for western redcedar. Obviously, the seeds that weigh most have the fewest SPG and vice versa. Seed orchard seeds in the Pinaceae are generally larger by approximately 15%, although differences exist between species, families, and years. In the Cupressaceae the difference in seed size between wild and seed orchard seeds appears negligible (Kolotelo 2000).

Seeds must have a purity of 97% and a moisture content between 4.9 and 9.9% for registration and use in Crown land reforestation

[3] The moisture content of wood is generally calculated on a dry weight basis (i.e., divided by dry weight versus fresh weight) to indicate the amount of water relative to the solid substance and can be greater than 100%. However, for seeds and many other living plant tissues, the weight of water is relative to the total weight of water + solid substance and lies between 0 and 100%.

The germination test is probably the test that first comes to mind when thinking of seed testing as it has immediate implications for seed quality and estimating the number of seedlings that can be obtained from a quantity of seeds. The germination test is based on four replicates of 100 seeds that have been treated with a specific protocol considered optimum, on average, for the species. The main features of a seed pretreatment protocol are the soak duration and the cold stratification duration. These are presented in Table 5 for conifers planted in BC. Cold stratification is thought to be most effective at temperatures between +2 and +5°C.

In a few species two protocols are initially used for germination testing, and the one producing the highest germination capacity is recommended for operational pretreatment and subsequent retesting. In particular, for lodgepole pine and the spruces, a soak only and a soak + stratification protocol are used as initial tests. The stratification treatment generally produces the higher germination capacity and rate, but problems with poor overall quality, mechanical damage, or fungi may result in the soak-only treatment being superior. ISTA recommends[4] double testing with a dry and a soak + stratify test, but this comparison confounds the effect of soaking and stratifying. Sowing dry seeds in the nursery is not recommended as the time required to uptake moisture in the seedling cavities will delay germination and probably decrease the uniformity of emergence. This will result in a greater input of energy during germination at the nursery.

...main features of a seed pretreatment are the soak duration and the cold stratification duration

Several species have specific modifications to the testing protocols due to complex dormancy mechanisms. For yellow-cedar, a 28 day period of warm stratification is also required to increase the germination capacity, although chemicals may be able to replace this warm stratification period (Xia and Kermode 2000). For Amabilis fir, subalpine fir, and Noble fir, a method which incorporates a split stratification regime, is generally implemented (Edwards 1986). This protocol includes four weeks of cold stratification at a high moisture content (≈45% = no surface drying of seeds) followed by surface drying the seeds to between 30 to 35% moisture content

[4] ISTA (International Seed Testing Association) is a large international body mainly concerned with the testing of agricultural seeds and when reasonable evidence is available indicating that we should deviate from their guidelines we may choose to do so. The AOSA (Association of Official Seed Analysts) guidelines are also used as a reference, but in general these two organizations have similar guidelines.

The germination test codes, associated species, and testing protocols employed at the BC Ministry of Forests Tree Seed Centre

Code	Species[a]	Soak (hours)	Stratification (days)	Additional protocol components	Germination[b] temp (°C)	Count days
G10	Douglas-fir, spruce, western larch	24	21		30–20	21
G20	Lodgepole pine, ponderosa pine	24	28		30–20	21
G31	Western hemlock, mountain hemlock	24	28		20–20	28
G32	Grand fir	48	28		30–20	28
G44	Amabilis fir, subalpine fir, noble fir	48	56		25–15	28
G52	Yellow-cedar	48	84 (28 warm/ 56 cold)	28 days at 20°C followed by 56 days at 2–5°C	30–20	28
G55	Western white pine	336	98		30–20	28
G64	Amabilis fir, subalpine fir, noble fir	48	84 (split)	28 days at ≈45% moisture followed by 56 days at 30–35% moisture	25–15	28
D1	Western redcedar	0	0		30–20	21
W1	Lodgepole pine, spruce	24	0		30–20	21

[a] Scientific names, common names, and abbreviations of BC conifer species are presented in Appendix 1.

[b] Eight hours at higher temperature with illumination and 16 hours at lower temperature in the dark.

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[b] Eight hours at higher temperature with illumination and 16 hours at lower temperature in the dark.

Figure 57 The contents of a germination test dish.

Figure 58 Germination testing a) environmentally controlled germinator and b) counting and removing germinants from dishes.

and placing the seeds back into cold stratification for an additional eight weeks. The majority of true firs mentioned here perform best with the split stratification regime, but a protocol involving 56 days of cold stratification without surface drying is also performed and the superior treatment for each seedlot is recommended for operational use.

Germination testing procedures vary slightly between facilities. The key is to have a well documented, reproducible regime that is based on accepted testing protocols (ISTA or AOSA). This guidebook will use the procedures employed at the BC Ministry of Forests Tree Seed Centre as an example.

Sowing dry seeds in the nursery is not recommended

For each of the four 100-seed replicates, the seeds will be soaked (except western redcedar) for the proper duration in a vial (Table 5). To prepare the germination box, place one layer of 22-ply kimpack in the dish, add 50 ml of water and compress the kimpack to evenly distribute water and provide a flat surface onto which you place one piece of filter paper (Figure 57). The water will be drained from the soaked seeds and they will be spaced equally onto the filter paper in the germination dish. The four germination dishes that encompass a stratified test will then be put into the cooler at 2°C for the appropriate stratification duration (Table 5). For yellow-cedar, the period of warm stratification is conducted at a constant 20° in a germination cabinet.

After the appropriate stratification period, the germination dishes are transferred to the germination cabinet (Figure 58) under conditions considered optimal. An assessment of germinated seeds will continue for up to 28 days and will be performed on Mondays, Wednesdays, and Fridays, with germinants counted and removed from the dish to estimate germination rate. A seed is considered germinated when its radicle is four times the length of the seed coat and no abnormalities are displayed by the germinant. The ISTA germination definition for tree species having epigeal germination is when the primary root and hypocotyl together exceed four times the length of the seed (ISTA 1999). Common abnormalities in conifers include reversed germinants (Figure 59a) and stunted radicles (Figure 59b). However, stunted hypocotyls and seeds in which the megagametophyte constricts the germinant (megagametophyte collar), not allowing germination to progress normally, are also seen. Abnormal germinants are recorded but not included in the estimate of germination capacity. In other regions of Canada the germination-vigour classes proposed by Wang (1973) are used to quantify germination capacity.

Abnormal germinants are recorded but not included in the estimate of germination capacity

Figure 59 a) Examples of reversed germinants, with cotyledons emerging first, in a variety of species and b) stunted radicles in Amabilis fir.

The germination capacity (GC) is the main criteria used to define seedlot quality. The GC is the percentage of seeds that have germinated during a germination test (21 or 28 days depending on species – see Table 5). Germination capacity is useful in quantifying seedlot quality and in determining the amount of seeds required to produce a given quantity of seedlings, but it should be supplemented with a variable describing the germination rate (faster germination usually equates to a more uniform crop).

The germination value (GV) has historically been used to define 'vigour' (Czabator 1962) and is the other variable in addition to GC that *was* available on SPAR. The GV has now been replaced by PV on SPAR. The GV is a product of two additional variables: mean daily germination (MDG) and peak value (PV) GV=MDG * PV. Figure 60 presents a graphical representation of GC, PV, and GV. The MDG is simply the germination capacity divided by the number of days in test [MDG=GC/#days in test]. For example, an interior spruce seedlot which is tested for 21 days, with a GC of 91% would have a MDG of 4.3. MDG is a linear description of germination, but germination is not linear and this parameter alone is not very useful. The PV is the point at which the cumulative germination percent divided by the number of days is maximum. The PV describes germination rate and is best understood with an example from a germination test sheet

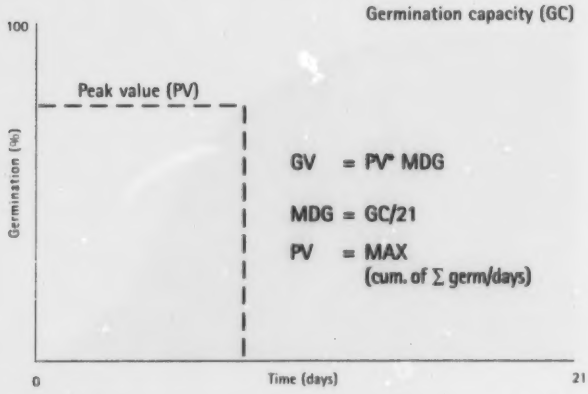

Figure 60 A graphical representation of germination capacity (GC), peak value (PV), and germination value (GV), also termed vigour.

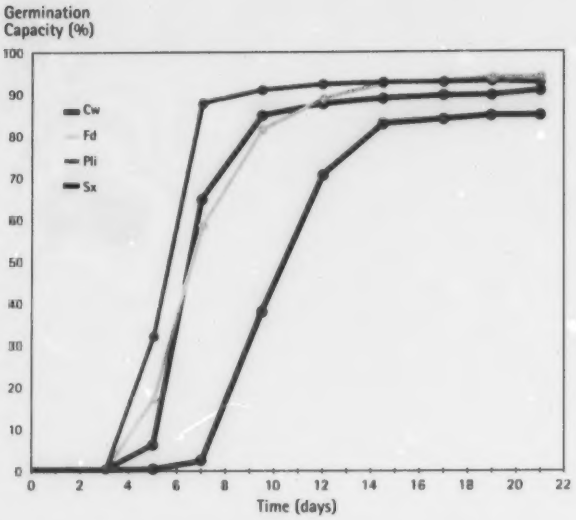

Figure 61 Representative germination curves for interior lodgepole pine (Pli), interior spruce (Sx), Douglas-fir (Fd), and western redcedar (Cw).

as illustrated in Table 6. The first step is to obtain the mean germination for each test date (i.e., for day 7: (52+57+55+60)/4 = 56). For each test date we will then calculate the average cumulative germination (i.e., for day 7: 20.8+56=76.8). The cumulative germination is then divided by the test days and the maximum value for the cumulative germination divided by test days is the peak value (i.e., for day 7: 76.8/7 =11.0). In this example, the peak value is 11.0 and it occurs on day 7. The PV is more informative if presented as cumulative germination and days to arrive at this level (77%/7 days) rather than simply a single number (11). The GC is equal to 91% and the MDG is equal to 4.3.

A comparison of the germination patterns for our four main species are illustrated in Figure 61. The high and rapid germination of lodgepole pine is typical of this species and aids in producing a uniform crop. The other end of the spectrum includes western redcedar which has a lower germination capacity and a more drawn out germination curve. This curve represents the testing of dry seed, but pelleting will move this curve even further to the right—delaying germination. Douglas-fir and interior spruce are similar and intermediate in their average germination patterns.

Table 6 The raw germination data used in the calculation of the germination parameters

Test Day	3	5	7	10	12	14	17	19	21
Rep	Number of Normal Germinants Counted								
1	0	20	52	10	8	0	0	0	0
2	0	21	57	10	4	2	0	0	0
3	0	23	55	8	5	1	0	0	0
4	0	19	60	7	3	1	2	0	0
Mean	0	20.8	56.0	8.8	4.0	1.0	0.5	0.0	0.0
Cumulative	0	20.8	76.8	85.5	89.5	90.5	91.0	91.0	91.0
Cum./Day	0	4.2	11.0	8.6	7.5	6.5	5.4	4.8	4.3

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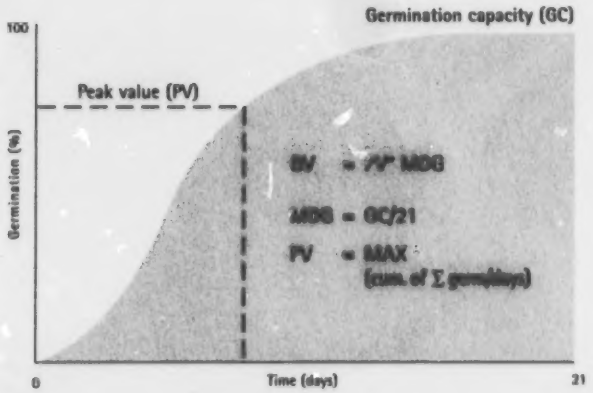

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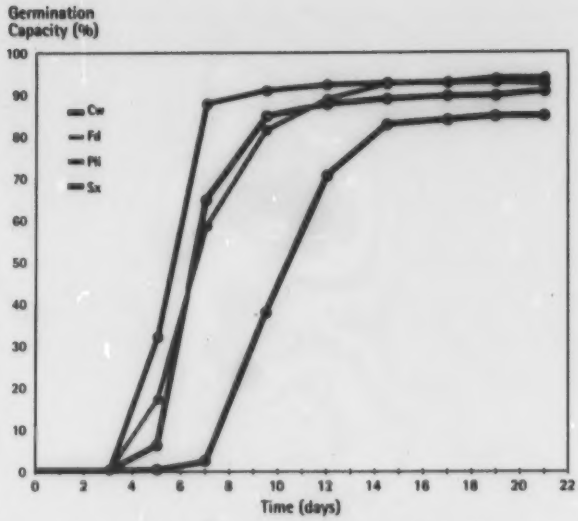

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	0	19	60	7	3	1	2	0	0
Mean	0	20.8	56.0	8.8	4.0	1.0	0.5	0.0	0.0
Cumulative	0	20.8	76.8	85.5	89.5	90.5	91.0	91.0	91.0
Cum./Day	0	4.2	11.0	8.6	7.5	6.5	5.4	4.8	4.3

This section focuses on the long-term storage of 'dry' conifer seeds, and presents some information on the storage of stratified seeds in relation to sowing. Species can generally be categorized as 'orthodox' or 'recalcitrant' in seed storage behaviour, although some authorities also include an intermediate class. Orthodox seeds, which includes conifers, can be dried to low moisture contents (<10%) and stored under sub-freezing temperatures for years, decades, or longer. Recalcitrant seeds cannot be dried down to low moisture contents and generally have very short life expectations (days, weeks, or months). The *Quercus* spp. (oaks) are an example of north-temperate recalcitrant species, although most recalcitrant species are tropical or sub-tropical in origin.

Long-term Storage

Long-term storability is generally quite good with conifer tree seeds, relative to many other orthodox plant species. It is generally accepted for orthodox species that storability, or the speed of deterioration, is related to storage temperature, storage moisture content, species, and initial seed quality. Storability is increased at low moisture contents and low temperatures and greatest for seeds with high initial seed quality. Harrington (1963) proposed a general rule of thumb which stated that the time for 50% of seeds to die (P50) was doubled for every 5°C reduction in temperature or 1% reduction in moisture level. As an example, if it takes 60 years to reach P50 at -20°C with a moisture level of 7%, the P50 age would be 30 years if the storage temperature was -10°C or if the moisture content was 8%. Much more sophisticated models have been developed, but they have mainly concentrated on agricultural crops. See the following references for a more thorough review of current research into storability (Roos 1989; Walters 1998).

Storability is increased at low moisture contents and low temperatures and greatest for seeds with high initial seed quality

At the BC Ministry of Forests Tree Seed Centre seeds are stored at -18°C and must be between 4.9 and 9.9% moisture content for registration. Seeds are stored in a plastic bag which has had all excess air expelled and then placed into a waxed cardboard box (Figure 62). An inventory location is placed on the cardboard box and the seedlot registration number placed inside and outside the plastic bag as well as on the box Figure 63). The freezer used for long-term storage should be secure from vandalism or fire and have a backup generator in case of power outage. Concerns have been raised that an increase in moisture content may occur when storage bags are opened under warmer conditions for seed withdrawal. A study of changes in moisture content during freezer storage indicated that the average change was approximately 0.1% per year, but that two species (Amabilis fir and western larch) had double this rate of moisture gain (Prabhu 1994). Seedlots of these two species should have moisture content retested after 10 years in storage.

Orthodox seeds can be dried to low moisture contents (<10%) and stored under sub-freezing temperatures for years, decades

Seeds may be stored in plastic, metal, or glass containers, but they should be airtight to avoid increases in moisture or infection from pests. A study in Scots pine using 14 kg plastic containers indicated that it takes 36 hours for a seed mass to warm from -16°C to an ambient temperature of 22°C (Sahlén and Bergsten 1982). This duration would be less in a smaller container (e.g., 7 kg bags used in BC), but it does illustrate that temperature change is not rapid. In Alberta the freezers are placed underground and the following advantages are stated: protection from fire, savings in maintenance costs, and savings in energy requirements through the natural insulation of soil around the building. After a mechanical

Figure 62 Long-term storage of tree seeds in polyethylene bags.

Figure 63 Long-term freezer storage facilities at the BC Ministry of Forests Tree Seed Centre.

failure of 72 hours, the temperature inside the freezers had only increased by 10°C (Altmann and Lafleur 1982).

There have been several reviews on the storability of conifer tree seeds (Barton 1954; Holmes and Buszewicz 1958; Wang 1974). The oldest tests are probably extremely conservative due to an incomplete understanding of the importance of moisture and temperature control and through improvements in tree seed collection and processing over time. Storability results of up to 50 years showed very slight changes in germination for slash pine although shortleaf pine deteriorated from 68 to 25% during the same period (Barnett and Pesacreta 1993).

In BC, some seedlots have been freezer-stored for more than 30 years. Several of these are presented in Figure 64 and include some of our oldest seedlots of coastal Douglas-fir, western hemlock, Sitka spruce, and western redcedar. Storability in coastal Douglas-fir is fairly good, but one can see that individual seedlots have unique storability patterns.

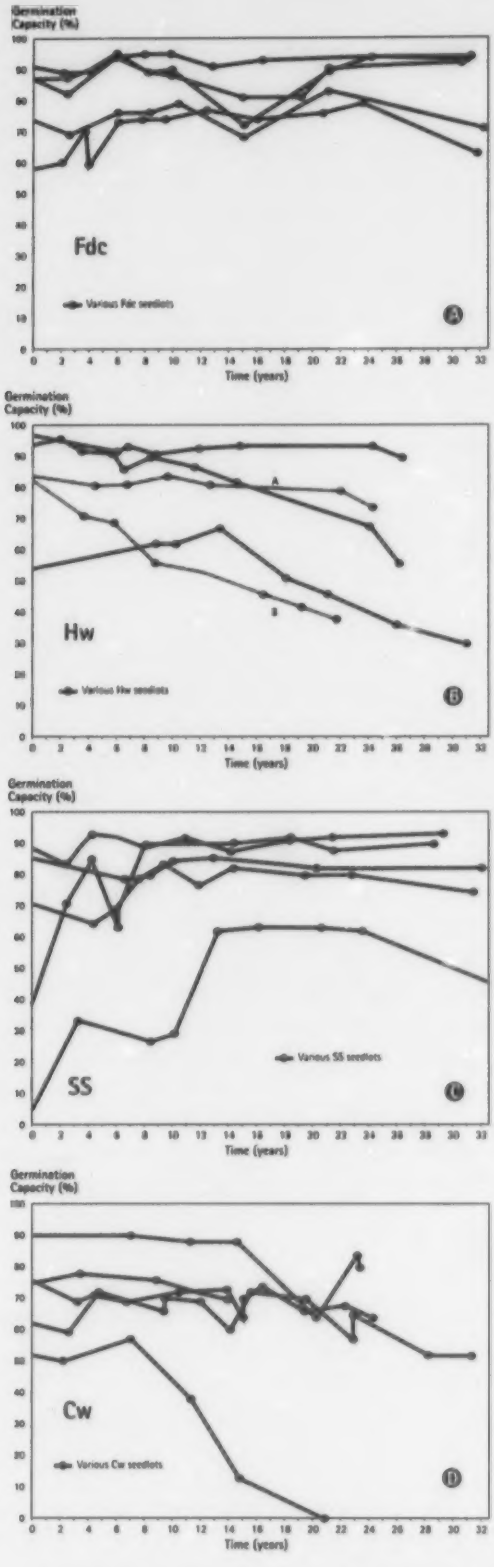

Figure 64 Long-term storability patterns at the BC Ministry of Forests Tree Seed Centre in seedlots of a) coastal Douglas-fir (Fdc); b) western hemlock (Hw); c) Sitka spruce (SS); and d) western redcedar (Cw).

While western hemlock seedlots A and B have an equivalent germination capacity going into freezer storage, after 20 years of storage their GC differs by approximately 40%! Initial seedlot quality is not always a good predictor of rate of deterioration.

In Sitka spruce some seedlots show an initially low GC, but over time increases in germination do occur. This is a perplexing issue and one for which we do not have an adequate explanation. Although controversial, it has been hypothesized that seedlots can become less dormant after prolonged freezer storage. However, Sitka spruce does not have deep dormancy and this does not adequately explain the large increases in germination found in some of the illustrated seedlots. Western redcedar is presented to illustrate the rapid deterioration (decrease in germination) that can occur and to show that some western redcedar seedlots can perform well after prolonged periods of freezer storage. These examples from our oldest seedlots illustrate that while species vary in storability, a greater source of variability may be the individual seedlot and how it has been handled leading up to freezer storage. Deterioration is not fully understood and, due to the long time periods involved, there have been few controlled experiments on seed deterioration in conifers.

The deterioration rate was calculated on a seedlot basis as the initial germination minus the current germination divided by the time in storage

The average species deterioration of tree seeds was quantified in BC to rank species and assign retesting frequencies. The deterioration rate was calculated on a seedlot basis as the initial germination minus the current germination divided by the time in storage based on a consistent test type and presented as change in germination capacity (GC) per year. The results and recommended retest frequencies for BC conifers are presented in Table 7. Western redcedar showed the highest deterioration rate (1.44%/year) among our species and is therefore retested more frequently than others. Some species exhibit positive deterioration rates and these values probably reflect sampling variation, improvements in seed testing, and possibly changes in dormancy due to storage rather than increased seed quality during storage. Seedlots that deteriorate more rapidly than the species average may be tested more frequently.

The actual cause of deterioration is not completely understood, but has attracted a great deal of research (mainly for agricultural crops and gene conservation purposes). It is generally accepted that seeds will first lose their vigour or ability to germinate under sub-optimal conditions, then lose the ability to germinate normally, and eventually die and not germinate at all. Suggested theories on seed deterioration include depletion of food reserves, alteration of chemical composition, membrane alteration, enzyme alteration, and genetic damage (Roos 1989).

Gene Conservation

Seed storage facilities can also play an important role in the conservation of genetic resources through the maintenance of a seed bank. This seed bank should contain a representative sample of the natural populations for the tree species of interest and be maintained strictly for conservation purposes. This form of gene conservation complements, but does not replace the need to have adequate areas of land dedicated to gene conservation within natural ecosystems. One of the biggest advantages of seed banks is efficiency as one handful (100 grams) of interior spruce seed can be composed of approximately 50 000 unique genotypes!

Table 7 Deterioration rate estimates and germination retest frequencies for BC conifers

Species	Deterioration rate (%/yr)	Retest frequency (months)
Amabilis fir	-0.78	24
Grand fir	-0.24	24
Subalpine fir	+0.67	24
Western redcedar	-1.44	18
Coastal Douglas-fir	+0.03	39
Interior Douglas-fir	-0.07	39
Mountain hemlock	-0.36	24
Western hemlock	-1.22	20
Western larch	-1.06	22
Coastal lodgepole pine	+0.08	42
Interior lodgepole pine	-0.01	36
Western white pine	-1.03	30
Ponderosa pine	-0.28	30
Sitka spruce	+0.10	42
Interior spruce	-0.07	36
Sitka x interior spruce hybrid	-0.25	30
Yellow-cedar	+0.46	36

While western hemlock seedlots A and B have an equivalent germination capacity going into freezer storage, after 20 years of storage their GC differs by approximately 40%! Initial seedlot quality is not always a good predictor of rate of deterioration.

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Western white pine	-1.03	30
Ponderosa pine	-0.28	30
Sitka spruce	+0.10	42
Interior spruce	-0.07	36
Sitka x interior spruce hybrid	-0.25	30
Yellow-cedar	+0.46	36

Storing Stratified Seed

At the nursery, prior to sowing, seeds should be maintained under stratification conditions (2–5°C) and given adequate aeration by opening the top of the bag when seeds arrive. These conditions will result in an extension of stratification, which may be beneficial, but seedlots of lower quality should be sown as soon as possible. Storage of stratified seeds occurs when sowing dates are delayed (and pretreatment has already been initiated) or following sowing with seeds remaining. If seeds are stored at high moisture contents for an extended period, germination or a reduction in viability can occur (Barnett 1974; Danielson and Tanaka 1978). If seeds are dried, unwanted germination can be avoided, storage can be greatly extended, and the pretreatment benefits (removal of dormancy) may be retained. Orthodox seeds should not be stored under sub-freezing temperatures at elevated moisture contents (above 14% moisture content), as ice crystals can form within the seeds, damaging contents and reducing germination (Barnett 1974; Bewley and Black 1994).

Orthodox seeds should not be stored under sub-freezing temperatures at elevated moisture contents

In Douglas-fir, drying from 45 to 35% moisture content removes moisture from the seed coat, but drying to 25% reduced moisture content in the seed coat, megagametophyte, and embryo (De Matos Malavasi et al. 1985). The moisture level for drying will probably vary by species, as those with more storage reserves (e.g., ponderosa pine or Douglas-fir) will probably store better than those with limited reserves (e.g., western redcedar or western hemlock). For loblolly pine, ponderosa pine, and Douglas-fir drying seeds to between 21 and 26% moisture content allowed the seeds to be stored for between 9 and 10 months without a significant reduction in germination. Seeds with a low GC will not retain viability as well as those with high GC values (Danielson and Tanaka 1978; Belcher 1982).

The BC Ministry of Forests Tree Seed Centre does not normally dry sowing requests if the sow date has been delayed by three weeks or less, but will dry sowing requests to approximately 20% moisture content if the delay is longer. If seed quality is poor the seed owner or nursery may decide to discard the seeds and initiate a new sowing request based on the revised timelines. Guidelines for the return of extra seeds from sowing requests are included in the seed sowing section.

Seed pretreatment refers to a group of activities carried out to better prepare seeds for sowing and germination. The activities include dormancy-breaking treatments that mimic the recommended test procedure, pelleting of western redcedar seed, sanitation procedures used to disinfest seeds of fungi, and other methods used to improve germination characteristics (i.e., uniformity). Details on seed sanitation are covered in a separate chapter. The major difference between testing and operational pretreatment is the quantity of seeds being treated. A variety of methods involving regulation of moisture uptake and the germination process (priming) are also reviewed here, although they have not received widespread operational use with conifers.

The process of **stratification** (imbibition followed by moist-chilling) is covered in detail due to its importance for overcoming dormancy and allowing germination to proceed. Stratification has mainly been described as a method to overcome embryo dormancy, but stratification may overcome coat-induced dormancy as well as offering other benefits. Many studies have indicated that stratification will improve the speed of germination and the uniformity of germination which are important considerations in producing a uniform seedling crop. The earlier in a crop cycle one introduces variability the more difficult it will be for the grower to correct this variability. Stratification also increases the 'vigour' of the seeds or their ability to germinate over a wide range of conditions (e.g., an expanded temperature range for maximum germination). The requirement for light in some species also seems to be overcome if conifer seeds are properly stratified. Recent evidence also indicates that natural repair mechanisms are activated during stratification (Wang and Berjak 2000). While stratification may be as close as one gets to a panacea in forestry, there are seedlots that perform better dry or with only a soak treatment. This is probably due to improper collection timing, mechanical damage to the seed coat, or fungal infection. Although seeds may be shipped dry to the nursery, it is recommended that they be soaked and surface dried prior to sowing when stratification is not recommended to facilitate rapid and uniform germination.

The effectiveness of stratification in overcoming seed dormancy requires that certain conditions be met: an appropriate moisture level, appropriate temperature, appropriate duration, and access to oxygen. If one of these elements is not adequately supplied, germination will be hampered through the incomplete removal of dormancy. Trial work on 10 seedlots of lodgepole pine and interior spruce stratified at moisture contents between 15 and 45% indicated that maximum germination occurred at 30% moisture content for both species (Hannam 1993). In white spruce, moisture contents below 20% were not adequate for stratification to be effective, while efficacy increased abruptly at 25% moisture content (Downie et al. 1998). The optimum stratification temperature falls between +2 and +5°C. Temperatures below freezing should be avoided as they are ineffective in breaking dormancy and may injure the imbibed seeds (Stokes 1965). Stratification at 5°C resulted in faster germination than at 2°C, for ponderosa pine and Douglas-fir, but if the higher end of this range is chosen one should frequently monitor the seeds as germination under stratification conditions is possible (Danielson and Tanaka 1978). No information is available on optimum oxygen levels, but the embryo and megagametophyte should have access to support respiration requirements of these tissues. Lack of oxygen will also promote the activity of anaerobic organisms that may contribute to seed deterioration, especially at elevated moisture contents and temperatures.

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Scarification (abrasion of seed coat), although important for many plant species, is not considered essential for germination in most conifers. Species in which scarification may prove beneficial are whitebark pine and western white pine (Pitel and Wang 1990; Hoff 1987). On an operational scale the procedure is difficult to perform effectively and efficiently in a repeatable manner. Although not widely used, a modified barley pearling machine has been suggested (Larsen 1925) as a suitable piece of equipment.

Seed pretreatment begins with the withdrawal of seeds from long-term storage (-18°C) (Figure 65). For sowing requests the amount of seed required is calculated based on BC Ministry of Forests sowing guidelines (see "Seed Sowing" chapter), but one can override these based on discussions between the seed owner and nursery. There is a significant difference between sampling for seed testing and operational seed pretreatment withdrawals for sowing. In testing, the sample taken to estimate germination is random and representative of a seedlot, but in operational seed preparation, seeds are withdrawn on an individual container basis. Logistics make it impossible to provide random, representative sampling for more than 5000 requests,

Figure 65 a) Withdrawing seeds from long-term storage and b) weighing pelleted western redcedar seeds for a sowing request.

primarily received within the few months that sowing occurs in BC. This is one area where differences between germination test results and germination of sowing requests can arise. A summary of all areas in which differences in germination results could occur is presented in the nursery results chapter.

Seed Soaking

Seed soaking is the first step in seed pretreatment for all species, except western redcedar. This step is also referred to as hydration or imbibition. As seeds gain moisture they become more susceptible to damage from mechanical impact, freezing, or high temperatures. Imbibed seeds should be handled very carefully to avoid damaging them.

Soak duration for operational pretreatment is equivalent to those given for testing in Table 8 (page 56), although actual procedures differ. Once seeds have been weighed and a label prepared, the seeds are placed into a nylon mesh net, which is tied and labelled. It is important not to tie the net too tightly around the seed mass as all seeds should have equal access to moisture for uniform uptake. The nets are placed into water baths of approximately 11°C that have a slow, but continuous flow (Figure 66). This 'running water soak' is intended to reduce the amount of seed-borne fungi on the seed coat through dilution and possibly aid in the removal of inhibitors in the seed coat (Martinez-Honduvilia and Santos-Ruiz 1978). For species with higher risks (i.e., coastal Douglas-fir or western larch) of significant pathogen levels, separate soaking compartments are used to avoid fungal cross contamination.

There is a significant difference between sampling for seed testing and with drawing seed for sowing requests

Running water soaks are applied to all sowing requests above 60 grams. After the soak the nets are withdrawn from the water bath and hung so that the excess moisture on and between the seeds drains away (Figure 67). For small sowing requests (<60 g), the seeds, without nylon mesh screens, are imbibed in labelled plastic bags filled with water. After these requests are soaked, run them over a screen to remove excess water and capture the seeds.

Seed soaking is the first step in seed pretreatment for all species, except western redcedar

The temperature of water used for seed hydration may affect the amount of moisture absorbed and possibly seed and seedling performance. It is generally accepted that at higher water temperatures imbibition will be faster, but Edwards (1971) found that if the imbibition period in Noble fir was extended past 14 days, a 5°C soak produced higher seed moisture content compared to a 25°C soak. Imbibition in

Figure 66 Placing sowing requests into a running water soak tank with a cover to ensure all seeds are submersed.

Figure 67 Draining seeds of extra moisture following the running water soak.

Pinus sylvestris was shown to vary by individual seeds and that a 10°C soak seemed to produce more uniform imbibition than at 5°C (Tillman-Sutela 1996). In Noble fir, a 48 hour soak at 4°C produced a reduction in germination capacity and this was attributed to the bulk pretreatment method, but no other soak temperatures were investigated (Jones et al. 1991). For loblolly pine, the imbibition temperature affected the moisture uptake rate, germination, and seedling development illustrating how a simple factor such as water temperature may have long ranging consequences in seedling production (Barnett and Vozzo 1985).

Surface Drying

Once draining is complete the seeds are surface dried with forced air and seed movement to facilitate uniform drying and then placed in cold stratification (Figure 68). Requests under 60 g are dried on blotter paper as forced air can dry these requests very quickly resulting in uneven drying or removal of internal moisture. A quick alternative to air drying is to surface dry seeds in a spin-drier (Gosling et al. 1994). Surface drying is carried out to optimize the moisture content for stratification, remove surface moisture to limit fungal growth, increase the speed at which oxygen may reach the embryo, and help the seeds to flow freely to assist mechanical sowing. When moisture is present on the seed coat the seeds stick together, complicating mechanical sowing. During surface drying it is important that only water from the surface of the seeds is removed and that drying does not remove internal moisture. This methodology is similar to the "target moisture content" prechill described by Jones and Gosling (1994) although the target level is reached through surface drying, gauged by visual and tactile cues. Tactile cues include seeds flowing freely through ones fingers and visually seeds are uniformly lighter in colour and duller in appearance as surface moisture is removed (see Figure 69).

Surface drying is carried out to optimize the moisture content for stratification, remove surface moisture to limit fungal growth, increase the speed at which oxygen may reach the embryo, and help the seeds to flow freely to assist mechanical sowing

Surface drying following at least partial stratification is advocated by some because maximum moisture content may not be obtained during the hydration period (Edwards 1996). Tanaka et al. (1986) do not recommend surface drying before stratification as germination speed may be reduced, but in this case a standard drying regime (25°C for 30 min.) was used without regard to drying rates. This resulted in the moisture content of surface dry seeds ranging between 26 and 41% for Engelmann spruce and lodgepole pine. If an inadequate amount of moisture is imbibed during the soak period either increase the soak duration or make moisture available during stratification.

Figure 68 Surface drying seeds a) illustrating a drying room that utilizes vents which draw moisture off the seed coat and b) a close-up of the manual movement of the seeds required for attaining uniform surface drying.

Controlling the moisture content during stratification can virtually eliminate the problem of pregermination during stratification, which has plagued many north-temperate conifers (Edwards 1986; Jones and Gosling 1994; Jensen 1996).

Excess moisture may also increase the bulking-up of seed-borne pathogens such as *Calosypha fulgens* which favours cool, moist conditions (Sutherland 1981). Higher moisture

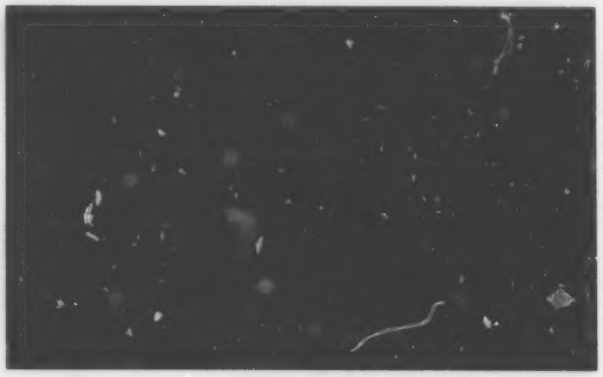

Figure 69 A comparison between surface moist (dark) and surface dry (light) seeds in interior spruce.

contents during stratification increases the respiration rate and this may be detrimental to subsequent seed performance due to the accelerated use of storage reserves (Leadem 1993). The seeds may use all of their reserves in respiration and other metabolic activities before they germinate and become self-sufficient. This concern is probably more an issue when sub-optimal conditions (e.g., temperature) or sowing delays occur. The issue of sowing delays deserved and received a great deal of attention when bareroot sowing was widespread in BC, but currently almost all crops are grown in containers and sowing delays because of weather conditions at the nursery are rare. If sowing delays are greater than three weeks the seeds should be dried to 20% moisture content to reduce metabolism and the nursery instructed to soak seeds for 24 hours prior to sowing. Clients are encouraged to update and maintain correct sowing dates on SPAR to allow for seed preparation to proceed as efficiently as possible.

The results of moisture content testing after stratification as part of the BC Tree Seed Centre Quality Assurance program (at time of shipping) are presented in Table 8. For example, in interior lodgepole pine 95% of the sampled requests had a moisture content between 30.0 and 30.6% (30.3 ± 0.3) over the past eight seasons. The Tree Seed Centre general target for stratified moisture content has been 30% and this coincides well with lodgepole pine and spruce, which account for approximately 75% of the sowing requests each season. The moisture contents in Table 8 are intended to quantify the differences in hydrated moisture content among species and are considered estimates of internal seed moisture content. It is suggested that our species fall into three classes for moisture content of seeds with fully hydrated internal components (embryo and megagametophyte):

Excess moisture may also increase the bulking-up of seedborne pathogens

Moisture Content	Species
Low [<30%]	Sitka spruce, western hemlock, and ponderosa pine
Medium [30 to 32%]	Interior spruce, interior lodgepole pine, coastal lodgepole pine, and Sitka × interior spruce hybrid
High [>32%]	Coastal Douglas-fir, Amabilis fir, mountain hemlock, western white pine, grand fir, interior Douglas-fir, western larch, and subalpine fir

At the BC Ministry of Forests Tree Seed Centre surface drying is not carried out on Amabilis fir, subalpine fir, Noble fir, western white pine, and yellow-cedar. However, free water between seeds within the seed mass is drained in these species. The *Abies* spp. are generally dried back after four

Table 8 Stratification moisture contents, number of requests, and confidence intervals for sowing requests at time of shipping sampled between 1992 and 2001

Species	Number of requests	Mean MC (%)	95% confidence interval (± value)
Amabilis fir	103	32.9	0.6
Grand fir	39	34.0	1.1
Subalpine fir	97	35.3	1.0
Coastal Douglas-fir	120	32.7	0.5
Interior Douglas-fir	148	35.0	0.5
Mountain hemlock	28	32.9	1.3
Western hemlock	102	26.7	0.7
Western larch	152	35.0	0.8
Coastal lodgepole pine	35	30.6	0.6
interior lodgepole pine	269	30.3	0.3
Western white pine	105	33.9	0.5
Ponderosa pine	96	27.8	0.4
Sitka spruce	55	25.8	1.2
Interior spruce	327	29.8	0.4
Sitka × interior spruce hybrid	38	30.2	1.0
	1714	31.5	

weeks stratification and given an additional eight weeks stratification similar to the stratification-redry treatment which has been shown to be advantageous (Edwards 1986; Edwards 1996; Leadem 1986; Tanaka and Edwards 1986). Tactile and visual cues are also used to gauge the dryback procedure and average dryback moisture content results are shown in Table 9. The other two species are not surface dried because of concerns with structures restricting water uptake to the megagametophyte and embryo (Hoff 1987; Tillman-Sutela and Kauppi 1998). Although yellow-cedar still receives a period of warm stratification the elimination of this procedure in western white pine greatly improved the quality of seeds shipped as mould buildup and pregermination were virtually eliminated (Figure 70).

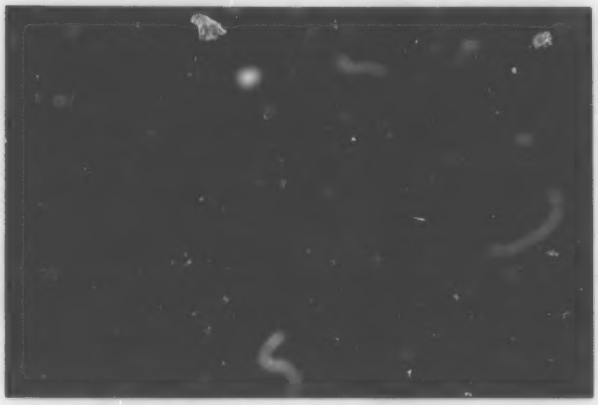

Figure 70 Problems associated with the former protocol of using warm stratification in western white pine: mould build-up and pregermination of non-dormant seeds in a seedlot.

Moisture content can be estimated non-destructively by weighing the stratified seeds and using target moisture content (knowing the initial request weight and storage moisture content) calculations. For example, if a sowing request is for 1351 grams at 7.9% MC and it weighs 1840 grams after surface drying, estimate the moisture content as follows:

Determine the ovendry weight of the request by using this MC equation:

ovendry weight = fresh weight * (1 – moisture content)
ovendry weight = 1351 * (1 – 0.079)
ovendry weight = 1244 g

Knowing the ovendry weight, calculate the moisture content at any weight using this MC equation:

$$\text{moisture content} = \frac{\text{fresh weight} - \text{ovendry weight}}{\text{fresh weight}}$$

moisture content = (1841 – 1244)/1841
moisture content = 0.324 or 32.4%

If unsure about the moisture status of a seedlot, or as part of a quality assurance program, use this simple method to determine MC non-destructively. Non-destructive moisture meters are available, but they perform poorly at moisture contents above about 12–15%.

Table 9 The number of dryback procedures and average moisture contents for *Abies* spp. after dryback

Species	Dryback (#)	Mean MC (%)
Amabilis fir	211	32.2
Subalpine fir	91	33.4
Noble fir	7	33.8

Stratification

In BC the technique used in cold stratification is referred to as 'naked stratification' as no media is included with the seeds during moist chilling. The term stratification was originally coined to describe the layering or 'stratification' of seeds between layers of moistened material. Material that has been used in stratification includes moistened cloth, sand, and peat moss. Sphagnum moss has been used in cold stratification as it has a high water-holding capacity and good aeration, and studies have also indicated reductions in the incidence of damping-off (Hess 1996; Wang et al. 1998). Moist chilling in polyethylene bags was initially investigated with loblolly pine (Hosner et al. 1959) and referred to as 'storage.' Both stratification and storage of moist seeds under cool conditions have been used to describe naked stratification, but due to its brevity, the term stratification has survived despite the lack of actual 'stratification' in the procedure.

Tactile and visual cues are used to gauge the dryback procedure

After surface drying the seeds are placed into a premoistened polyethylene bag of appropriate size for the request, a label is attached and the top is tied to keep the top of the bag open approximately 3 cm. Polyethylene bags up to 4 mil (0.102 mm) allow for some oxygen and carbon dioxide exchange, but it is recommended to have the top of the bag open to allow for additional aeration (Figure 71). In some facilities air is piped into the bag through a plastic tube. For sowing requests of all species except western white pine, a maximum of 3000 grams is placed into each poly-bag. For example, if a sowing request is 4800 grams two bags, and two labels, will be used and they will both contain 2400 grams of seeds. Due to difficulties in obtaining

In BC the technique used in cold stratification is referred to as 'naked stratification' as no media is included with the seeds during moist chilling

contents during stratification increases the respiration rate and this may be detrimental to subsequent seed performance due to the accelerated use of storage reserves (Leadem 1993). The seeds may use all of their reserves in respiration and other metabolic activities before they germinate and become self-sufficient. This concern is probably more an issue when sub-optimal conditions (e.g., temperature) or sowing delays occur. The issue of sowing delays deserved and received a great deal of attention when bareroot sowing was widespread in BC, but currently almost all crops are grown in containers and sowing delays because of weather conditions at the nursery are rare. If sowing delays are greater than three weeks the seeds should be dried to 20% moisture content to reduce metabolism and the nursery instructed to soak seeds for 24 hours prior to sowing. Clients are encouraged to update and maintain correct sowing dates on SPAR to allow for seed preparation to proceed as efficiently as possible.

The results of moisture content testing after stratification as part of the BC Tree Seed Centre Quality Assurance program (at time of shipping) are presented in Table 8. For example, in interior lodgepole pine 95% of the sampled requests had a moisture content between 30.0 and 30.6% (30.3 ± 0.3) over the past eight seasons. The Tree Seed Centre general target for stratified moisture content has been 30% and this coincides well with lodgepole pine and spruce, which account for approximately 75% of the sowing requests each season. The moisture contents in Table 8 are intended to quantify the differences in hydrated moisture content among species and are considered estimates of internal seed moisture content. It is suggested that our species fall into three classes for moisture content of seeds with fully hydrated internal components (embryo and megagametophyte):

Excess moisture may also increase the bulking-up of seedborne pathogens

Moisture Content	Species
Low [<30%]	Sitka spruce, western hemlock, and ponderosa pine
Medium [30 to 32%]	Interior spruce, interior lodgepole pine, coastal lodgepole pine, and Sitka × interior spruce hybrid
High [>32%]	Coastal Douglas-fir, Amabilis fir, mountain hemlock, western white pine, grand fir, interior Douglas-fir, western larch, and subalpine fir

At the BC Ministry of Forests Tree Seed Centre surface drying is not carried out on Amabilis fir, subalpine fir, Noble fir, western white pine, and yellow-cedar. However, free water between seeds within the seed mass is drained in these species. The *Abies* spp. are generally dried back after four

Table 8 Stratification moisture contents, number of requests, and confidence intervals for sowing requests at time of shipping sampled between 1992 and 2001

Species	Number of requests	Mean MC (%)	95% confidence interval (± value)
Amabilis fir	103	32.9	0.6
Grand fir	39	34.0	1.1
Subalpine fir	97	35.3	1.0
Coastal Douglas-fir	120	32.7	0.5
Interior Douglas-fir	148	35.0	0.5
Mountain hemlock	28	32.9	1.3
Western hemlock	102	26.7	0.7
Western larch	152	35.0	0.8
Coastal lodgepole pine	35	30.6	0.6
interior lodgepole pine	269	30.3	0.3
Western white pine	105	33.9	0.5
Ponderosa pine	96	27.8	0.4
Sitka spruce	55	25.8	1.2
Interior spruce	327	29.8	0.4
Sitka × interior spruce hybrid	38	30.2	1.0
	1714	31.5	

weeks stratification and given an additional eight weeks stratification similar to the stratification-redry treatment which has been shown to be advantageous (Edwards 1986; Edwards 1996; Leadem 1986; Tanaka and Edwards 1986). Tactile and visual cues are also used to gauge the dryback procedure and average dryback moisture content results are shown in Table 9. The other two species are not surface dried because of concerns with structures restricting water uptake to the megagametophyte and embryo (Hoff 1987; Tillman-Sutela and Kauppi 1998). Although yellow-cedar still receives a period of warm stratification the elimination of this procedure in western white pine greatly improved the quality of seeds shipped as mould buildup and pregermination were virtually eliminated (Figure 70).

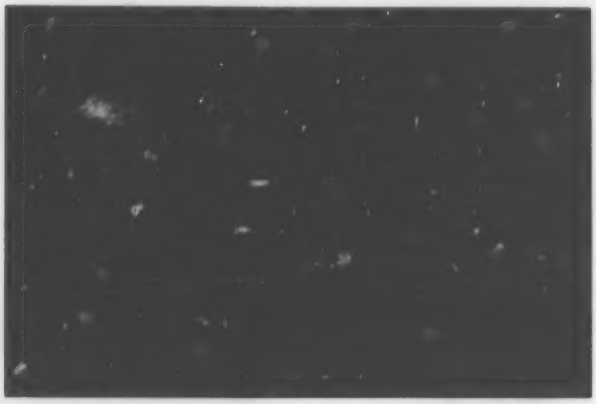

Figure 70 Problems associated with the former protocol of using warm stratification in western white pine: mould build-up and pregermination of non-dormant seeds in a seedlot.

Moisture content can be estimated non-destructively by weighing the stratified seeds and using target moisture content (knowing the initial request weight and storage moisture content) calculations. For example, if a sowing request is for 1351 grams at 7.9% MC and it weighs 1840 grams after surface drying, estimate the moisture content as follows:

Determine the ovendry weight of the request by using this MC equation:

ovendry weight = fresh weight * (1 – moisture content)
ovendry weight = 1351 * (1 – 0.079)
ovendry weight = 1244 g

Knowing the ovendry weight, calculate the moisture content at any weight using this MC equation:

$$\text{moisture content} = \frac{\text{fresh weight} - \text{ovendry weight}}{\text{fresh weight}}$$

moisture content = (1841 – 1244)/1841
moisture content = 0.324 or 32.4%

If unsure about the moisture status of a seedlot, or as part of a quality assurance program, use this simple method to determine MC non-destructively. Non-destructive moisture meters are available, but they perform poorly at moisture contents above about 12–15%.

Table 9 The number of dryback procedures and average moisture contents for *Abies* spp. after dryback

Species	Dryback (#)	Mean MC (%)
Amabilis fir	211	32.2
Subalpine fir	91	33.4
Noble fir	7	33.8

Stratification

In BC the technique used in cold stratification is referred to as 'naked stratification' as no media is included with the seeds during moist chilling. The term stratification was originally coined to describe the layering or 'stratification' of seeds between layers of moistened material. Material that has been used in stratification includes moistened cloth, sand, and peat moss. Sphagnum moss has been used in cold stratification as it has a high water-holding capacity and good aeration, and studies have also indicated reductions in the incidence of damping-off (Hess 1996; Wang et al. 1998). Moist chilling in polyethylene bags was initially investigated with loblolly pine (Hosner et al. 1959) and referred to as 'storage'. Both stratification and storage of moist seeds under cool conditions have been used to describe naked stratification, but due to its brevity, the term stratification has survived despite the lack of actual 'stratification' in the procedure.

Tactile and visual cues are used to gauge the dryback procedure

After surface drying the seeds are placed into a premoistened **polyethylene** bag of appropriate size for the request, a label is attached and the top is tied to keep the top of the bag open approximately 3 cm. Polyethylene bags up to 4 mil (0.102 mm) allow for some oxygen and carbon dioxide exchange, but it is recommended to have the top of the bag open to allow for additional aeration (Figure 71). In some facilities air is piped into the bag through a plastic tube. For sowing requests of all species except western white pine, a maximum of 3000 grams is placed into each poly-bag. For example, if a sowing request is 4800 grams two bags, and two labels, will be used and they will both contain 2400 grams of seeds. Due to difficulties in obtaining

In BC the technique used in cold stratification is referred to as 'naked stratification' as no media is included with the seeds during moist chilling

Figure 71 Seeds in stratification: a) a view of sowing requests in stratification and b) illustrating the open bag for increasing oxygen exchange.

high germination with western white pine the maximum bag size is set at 1000 grams to allow for additional aeration and closer observation of the seeds during stratification. Optimization of stratification methods are currently being investigated for western white pine. During stratification monitoring of seed condition is important and may be accompanied by the gentle massaging of the bags or rotating the seeds to ensure anaerobic conditions do not build up in part of the bag, especially with species requiring long stratification (Figure 72).

Seeds are shipped to nurseries following the appropriate pretreatment, unless the nursery indicates otherwise. Bags of seeds are closed to avoid contamination during transport. Open them again upon arrival. Stratified seeds are fragile and must be handled with care. Seeds are shipped overnight, by courier, in expanded polystyrene containers with insulating packing material covering the seeds and ice packs placed on top to help maintain a cool temperature during shipping (Figure 73). With each shipment the seeds shipping label is attached indicating the simple steps for properly handling seeds at the nursery (Figure 74).

Bags of seeds are closed to avoid contamination during transport. Open them again upon arrival

Pelleting

Pelleting is primarily used in forestry to aid in the sowing of small, light, winged, or irregularly shaped seeds. In BC all western redcedar seeds are pelleted with a mixture of diatamaceous earth and binders that are slowly bound to the seeds with a water mist in a rotating drum (Figure 75). Western redcedar is not soaked or stratified prior to sowing thus allowing the pelleting process to be performed on dry seeds. Some nurseries are also requesting that red alder (*Alnus rubra* Bong.) seed be pelleted. Imbibed seeds can also be pelleted, but the shelf life will be greatly reduced. The disadvantage of pelleting is the reduction in the germination rate as the radicle must penetrate the pellet as well as the seed coat. Western redcedar has limited megagametophyte reserves, and under suboptimal conditions, one risks these reserves being used before germination is complete. Germinate pelletized western redcedar as quickly as possible, but do not allow the pellet to dry out as it may become "cemented" to the seed, restricting cotyledon emergence. The delay that the pellet imposes on the seeds has been estimated at four days for western redcedar. Pelleting is performed solely to be able to efficiently sow the seeds into containers.

Pelleting was shown to have a negative impact on germination in white spruce, jack pine, and red pine, but did not influence germination in black spruce. If black spruce was sown at sub-optimal germination temperatures, germination was adversely affected by pelleting. Pelleted black spruce can be stored for three years without adverse effect on germination (Fraser and Adams 1980).

Figure 72 Monitoring of seeds during cold stratification.

Figure 73 Contents of a seed shipment a) including ice packs and insulating material and b) a seed shipment awaiting pickup in the cooler.

Other Seed Treatments

These other seed treatments may have their place in the conifer seed handling system, but experience with their use is limited thus far. These techniques have flowed from agricultural use and are mainly aimed at imbibing seeds to a moisture content that allows germination to progress without radicle emergence. Several differences between sowing in agricultural crops and conifers are worth noting. Conifers are generally soaked and stratified prior to sowing while most agricultural seeds are sown dry. Agricultural crops are mainly sown into fields with relatively unpredictable germination conditions while conifers are mainly sown in greenhouses allowing much greater control of environmental conditions. Agricultural seeds are genetically quite uniform within a variety while conifer seeds are still very diverse. The optimization of any tree seed treatments will be more complicated than agricultural crops because of this level of diversity. These differences indicate that while these latter seed treatments are well suited for agricultural crops, a great deal of additional work is required for them to be used operationally with conifers.

Figure 74 Shipping label attached to sowing requests indicating proper treatment of seeds upon arrival.

The following are 'newer' techniques used to prepare seeds for germination. Seed priming is used to describe a wide variety of methods to partially hydrate seeds to a moisture content that initiates germination processes (biochemical reactions), but does not allow radicle emergence to occur. These methods have been used mainly with agricultural crops, but some experimental work with conifers is occurring. A brief definition of common methods and their many synonyms is presented to clarify the nomenclature present in the literature. For additional information on priming, refer to the excellent reviews by Heydecker and Coolbear (1977) and Welbaum et al. (1998).

Hydro-priming (also hardening[5]) involves controlling the amount of water entering seeds through a reduction in soaking time, exposure to low temperature, or treatment under high humidity conditions. This is similar to the target moisture content concept (Jones and Gosling 1994), although with many agricultural crops moist chilling is not performed.

Osmotic priming (also osmotic conditioning) involves the use of an **osmoticum**, such as **polyethylene glycol** to control moisture uptake through the reduction in the **water potential** of the solution (see page 85 for a more complete explanation of water potential). An osmoticum with a high molecular weight is favoured as it does not penetrate the seeds. Seeds

5 In some references hardening is used to describe repeated wetting and drying cycles that have been associated with drought resistance. A good review is provided in Heydecker and Coolbear (1977).

Figure 75 Pelleting of western redcedar.

are usually washed and dried back to the previous storage moisture content. The disadvantages of this method are the need to determine the optimum relationship between osmotica used, concentration, and temperature, which will probably vary by species and possibly seedlot. There is also a need to dispose of the priming solutions, which has proved to be operationally problematic.

Solid matrix priming (SMP) involves the use of a solid carrier with the seeds and water which are incubated together to generate a negative water potential. Examples of solid carriers include vermiculite, sand, calcited clay, and spagnum moss. After incubation the mixture is dried and seeds separated from the media using conventional seed processing equipment. Although many studies have indicated the superiority of SMP over osmotic priming there is still a large amount of optimization to be performed beforehand and some procedures are patented, but disposal is generally considered an easier activity with SMP.

Agricultural seeds are genetically quite uniform within a variety while conifer seeds are still very diverse

Drum priming (also invigoration) involves the use of a horizontal rotating drum into which water vapour is released until a predetermined level of hydration is reached. Drum priming is gaining in popularity over the above methods as large quantities of seeds can be treated operationally and there are no associated waste materials as found in the above two methods.

Biopriming is a method that may be combined with any of the above priming treatments and involves the introduction of beneficial organisms to reduce disease incidence or improve other aspects of germination. Fungicides may also be applied in combination with biopriming.

Fluid drilling refers to various means in which pre-germinated seeds are sown either in a protective gel or delivered to the soil with an amount of water. This is not strictly a priming treatment, but a sowing or delivery system. The gel or water delivered with the seeds may also contain nutrients, plant growth regulators, or pesticides (Gray 1981).

The topic of hydropriming or simply soaking seeds often raises the issue of aeration and whether this needs to be incorporated into the procedure. For Ocala sand pine it was shown that germination improved by using a 24 hour aerated soak (Outcalt 1991). In a trial with BC species the effects of aeration were not consistent across species. The practice produced improvements in western hemlock, western redcedar, and older seedlots of pine and spruce. Both coastal and interior Douglas-fir were negatively affected by aeration. Even within species the effectiveness was seedlot specific indicating difficulties in applying the procedure on an operational scale (Boomhower 1995).

Osmotic priming has been tried with several conifers with varying degrees of success. Huang (1989) found that all treatments enhanced seedling uniformity and reduced the number of abnormal seedlings, but many treatments resulted in decreased germination capacity for lodgepole pine and white spruce. Unfortunately no comparisons with stratification were included. Reduction in germination capacity, but improvements in germination speed were found in *Pinus brutia* var. *eldarica*. The authors state 'other invigoration treatments such as stratification or controlled hydration may offer greater benefits in nursery production at lower cost' (Khalil et al. 1997, page 24). In northern Fennoscandia osmotic priming was used to replace physiological ripening of Scots pine as complete seed maturation often does not occur at these latitudes. Improvements in germination capacity and seed vigour occurred when seeds were collected after anatomical maturity, but before physiological maturity (Sahlén and Wiklund 1995).

Solid matrix priming was investigated in loblolly pine using clay as the solid carrier. Increases in germination rate and synchrony were observed, but only slight improvements in germination capacity were observed. The optimum treatment reduced differences in germination vigour among families (Wu et al. 1999). Membrane tube invigoration (similar to drum priming) was shown to be far superior to osmotic priming for white spruce and jack pine. The invigoration regime produced faster germination, without sacrificing capacity, compared to prechilling, but all seedlots chosen were considered non-dormant (Downie et al. 1993).

Nurseries in BC are increasingly aware of the importance of sanitation in combating pathogens that have repeatedly caused significant stock losses. While there can be a large microflora associated with seeds, their effects on seed viability and seedling production are not well understood (Richardson 1996; Mittal and Wang 1993). Therefore, it is often critical to reduce the sources and degree of pathogen inoculum on seeds. Disinfesting seed can control some seedborne fungi. The first fungus understood to be seedborne was *Tilletia caries*, responsible for causing bunt or covered smut of wheat (Tull 1733). Tull recorded observations of farmers whose wheat seed that had been salvaged from the ocean was free of smut, concluding that the brining action of the salt water disinfested the wheat seed. Early in the drive to guarantee high quality seed, organizations like ISTA recognized the need to better characterize seedborne pathogens. Latest estimates document approximately 1300 seedborne organisms capable of being pathogenic. Most are fungi; seedborne fungi have been reported on 305 host genera in 96 plant families (Richardson 1996). In addition to pathogens, many saprophytic fungi can infect seeds when they are harvested and stored under sub-optimal conditions. They sometimes reduce seed viability and produce mycotoxins (e.g., aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*).

Strategies for Fusarium *and* Caloscypha *control are aimed at minimizing the ability of each pathogen to spread within a seedlot, while methods to control* Sirococcus *infection are designed to eliminate the organism*

Some seedborne fungi can spread to clean seeds when they are stored with contaminated or infected seeds in a moist environment at temperatures conducive to fungal growth. These temperatures vary for fungal species but in general range between 15–22°C. In some of these situations, control measures can be as simple as avoiding favourable conditions for fungal growth. Where conditions conducive to fungal growth and spread cannot be avoided, more aggressive measures can be taken to reduce inoculum levels. In BC, most efforts have been concentrated on assaying seedlots for *Caloscypha*, *Sirococcus*, and *Fusarium*. When seedborne *Caloscypha fulgens* and species of *Fusarium* begin to infect or contaminate seeds in a seedlot, steps should be taken to minimize the impact of these organisms (5% or more for *Caloscypha* and *Fusarium* or 1% for *Sirococcus*).

Strategies for *Fusarium* and *Caloscypha* control are aimed at minimizing the ability of each pathogen to spread within a seedlot.

The methods used to control the impact of *Sirococcus* infection in a seedlot are designed to eliminate the organism. Seeds infected with *Sirococcus* usually develop *Sirococcus* blight, where primary needles on germinants die from the base upwards. The importance of this seed-borne disease lies in its ability to kill seedlings via secondary spread of inoculum from infected germinants.

Research into seedborne pathogens indicated that early stages of fungal colonization of seeds are dependent primarily on abiotic factors such as water availability and temperature (Ramos et al. 1997). Because most of these fungi contaminate only seed coat surfaces, they depend more on environmental relative humidity than seed moisture content (Thomsen and Schmidt 1999). Successful pathogens exploit the best opportunities to infect seed. For example, they may be transported along with seeds of the host plant, capable of causing infection at the earliest opportunity (Richardson 1996).

Any proposed strategy to curtail the effects of seedborne pathogens must be practical. There is little value in telling a seed processor or nursery grower that seeds are infected/infested unless it is possible to provide assay information on the effects of the infection on seedling requests and whether or not any treatment or strategy is available. In addition, there is also an ongoing need for knowledge of the disease process and how to make fungal assay results meaningful tools of what will happen to seeds once they are sown.

There are three general strategies to minimize losses from seedborne pathogens (Berger 1977). These are eliminating or reducing initial inoculum, slowing the rate of pathogen spread, and shortening the exposure time of the seed to the pathogen. Cone collection protocols, seed orchard management, and seed processing fall into the first category and are operationally referred to as sanitation. Seed treatments and storage relate closely to the second strategy, while stratification and germination procedures are encompassed by the third. Seed treatments fall into three basic categories: *seed protection* (e.g., chemical, biological, physiological

procedures that protect the seed/seedlings from pathogens); *seed disinfestation* (e.g., removal of seed coat pathogens); and *seed disinfection* (e.g., eliminate pathogens inside the seed) (Bennett et al. 1991). Ideally any treatment should reduce or eliminate the effects of the pathogen but not adversely affect seed germination.

Littke (1996) indicated that an understanding of the source and development of seedborne fungi was needed to reduce losses and realize gains in the nursery. He surmised that the most likely modes of seed contamination are physical transfer from the exterior of the cone to the seed coat during seed development, cone storage, and seed cleaning; cross contamination with "dirty" seedlots during seed processing, imbibition, stratification, and sowing; and contact with soil during storage or collection from squirrel caches. Overall, he concluded that 90% of seedborne inoculum resides on the seed coat and that cone-borne fungi are the most likely source of infestation. Cone handling and seed storage protocols that enhance drying and sanitation can reduce fungal contamination levels. Seed treatments (e.g., hydrogen peroxide, bleach, or bromine) are effective only in reducing levels of infestation and can have both positive and negative effects on germination performance (Littke 1996).

...three general strategies to minimize losses from seedborne pathogens – eliminating or reducing initial inoculum, slowing the rate of pathogen spread, and shortening the exposure time of the seed to the pathogen

Seed Cleaning Techniques

Campbell and Landis (1990) developed a strategy for dealing with seedborne diseases by determining the degree of possible risk to a nursery manager and attaching appropriate actions to minimize their effects on container nursery production. The general methods listed for treating seeds were surface cleaning or washing with running water; surface disinfecting seed coats with heat or chemical disinfectants; and coating seeds with fungicides (e.g., thiram or captan). They advocated a sequential approach to managing seed diseases. The steps are:

1. Determine if seed treatments are warranted. Treatments should be considered if the seeds fit any two of the following criteria: a) seedlings are to be grown in containers; b) the seedlot has a history of problems; c) the seeds are from a high risk conifer species; and d) the seedlot is of high value (e.g., seed orchard seedlots).
2. Assay the seedlot and identify the pathogens. Most seedlots do not possess significant levels of pathogens. Fungal assay results are present on sowing request labels (Figure 33, page 31) and on SPAR.
3. Treat assayed seedlots with significant contamination by cleansing seed surfaces (e.g., running water soaks and disinfecting seed surfaces with bleach or hydrogen peroxide, for example).
4. Surface dry seeds during extended stratification.

This section of the guide will primarily concentrate on the third step.

Once the decision has been made that there is a risk to seeds, procedures are needed to reduce the potential for seedborne pathogens to adversely affect seed viability and seedling survival and health. For the most part, nearly all techniques reduce seed coat infestations. Unfortunately, most of these seed cleaning techniques have little effect on seedborne *Caloscypha* and *Sirococcus*. For these two pathogens recommendations include reducing stratification periods if possible, sowing the seedlot as soon as possible after stratification, providing optimal germination conditions, and reducing the number of seeds per cavity by seedlot upgrading techniques (Landis et al. 1990; Sutherland et al. 1989).

Most seed treatment research has focused on reducing seed coat infestations of *Fusarium* spp. (Axelrood et al. 1995; James 1985). Presence of *Fusarium* on seeds and/or seedlings may be an unsatisfactory indicator of disease potential (James et al. 1989a, 1987). Causal relationships are difficult to pinpoint and some researchers question the true effects of seed treatments. Under operational conditions, any technique that can reduce the *Fusarium* disease potential helps reduce possible risks of disease. This does not preclude the notion that micro-environmental conditions may still be favourable to disease expression, but the degree of damage may be lessened with the reduced number of potential infection vectors. Research has shown that the relation of contamination level and subsequent disease may vary greatly among seedlots (James 1985). For Douglas-fir and ponderosa pine, James (1987a) found that most of the seedlots he tested had less than 10% seedborne contamination with *Fusarium*. Although infestation levels appear low, they may be sufficient to cause widespread disease. This may be related to recent observations that infestation levels of *Fusarium* spp. can increase greatly during seed imbibition and stratification (Axelrood et al. 1995; Hoefnagels and Linderman 1999). Neumann (1996, 1997) found that *Fusarium* levels on seedlots of Douglas-fir, spruce, western larch, and true firs increased substantially during stratification even on seedlots with low (<1%) dry seed pathogen levels. Numerous pathogenicity assays on conifer seeds, particularly Douglas-fir, have demonstrated a high

...infestation levels of Fusarium *spp. can increase greatly during seed imbibition and stratification*

degree of virulence for a majority of the isolates of selected *Fusarium* species (Axelrood et al. 1995; James et al. 1989a, 1989b, 1987). It must be emphasized that many of these pathogenic *Fusarium* species were retrieved from asymptomatic seedlings suggesting that environmental conditions play a key role in disease expression (Motta et al. 1996).

Numerous methods have been suggested or evaluated in an effort to reduce or eliminate seedborne contamination. One method has been to apply fungicides directly to the seed coats (Bennett et al. 1991). A second method has involved rinsing seed in running water for 24–48 hours to wash off fungal spores (James 1987b; James and Genz 1981). A third method recommends soaking seed for a determined time period in sterilants (e.g., sodium hypochlorite, ethanol, or hydrogen peroxide) to disinfect seed coats (Dumroese et al. 1988). These and other methods such as biological control will be explored in greater detail.

Running Water

Background

One of the easiest recommended strategies is running water imbibition coupled with a post-stratification rinse with running water. From a disease management perspective, the practical benefit of running water versus standing water soaks is the removal of fungal inoculum from infested seed coats (James and Genz 1981; James 1987a). In most studied seedlots, *Fusarium* levels have been observed to increase during stratification though inoculum levels are less in running water compared to standing water soaks. The result of running water treatment is an overall reduction in post-stratification seedborne levels of *Fusarium* (Axelrood et al. 1995; James 1985; Dumroese et al. 1988; Neumann, 1993).

Methodology

At the BC Ministry of Forests Tree Seed Centre, the method has been to pre-treat sowing requests in a mesh bag in a tank of running water for 24–48 hours. Unfortunately, this type of treatment requires extensive water resources, which may be limited at some facilities. It is suggested that even systematic water exchanges are sufficient to conserve water and reduce the amount of seed coat infestations. Complete water exchanges every 4–8 hours will have similar benefits. The addition of supplemental aeration between exchanges is recommended. The associated water movement helps to dislodge seedcoat fungi while maintaining an even distribution of oxygen within the water column.

The seeds making up a sowing request should be placed in a running water volume at least three times the seed volume within a mesh or porous container to allow for sufficient coverage of seed surfaces. The temperature of the water should not be higher than 25°C (Kaufmann and Eckard 1977). After stratification, some studies have recommended at least an additional 48 hour rinse (Dumroese et al. 1988). This may have the effect of removing fungal inoculumm on the seed coats that was established during stratification. Figures 76 and 77 (a and b) demonstrate the effect of running water on removing some of the seedborne fungal mycelium on heavily infested seeds of *Abies lasiocarpa* and *Picea glauca*.

It is the general practice at the Tree Seed Centre to soak together sowing requests of most seedlots and conifer species, such as interior spruce and lodgepole pine, in large tanks for the running water soak period. However, certain species such as Douglas-fir and western larch, and seedlots with critical levels of fungi are now imbibed in individual running water tanks (see Figure 78, page 65). Some operational factors may contribute to increases in *Fusarium* inoculum such as cross-contamination from infested to uninfested seedlots and sowing request size.

...the practical benefit of running water versus standing water soaks is the removal of fungal inoculum from infested seed coats

Neumann (1995) identified the potential for cross-contamination problems between low and highly infested seedlots of Douglas-fir and western larch when several seedlots are soaked together (see Figure 79, page 65). In 1996, she found significant differences in *Fusarium* levels between seeds sampled from the centre and edges of seed bags from three sizes of Douglas-fir requests (100 g, 250 g, and 500 g) though no significant trend was found.

Hydrogen Peroxide

Background

By far, the most common chemical seed sanitation treatment is hydrogen peroxide. Substantial differences in concentration, duration of treatment, stratification timing, and conifer species tolerance have been reported by a variety of researchers. A summary of published hydrogen peroxide treatment results is listed in Table 11 (page 66).

Figure 76 Seedborne fungi on spruce seeds a) prior to and b) after a post-stratification running water rinse. c) The effect of a post-stratification soak in a 3% hydrogen peroxide solution for 4 hours on seedborne fungi.

Figure 77 *Caloscypha* on *Abies lasiocarpa* seeds a) prior to and b) after a post-stratification running water rinse. c) The effect of a post-stratification soak in a 3% hydrogen peroxide solution for 4 hours on seedborne fungi.

Most of these studies result in two general treatment categories: 30% hydrogen peroxide for under 1 hour; or a 3% solution for over 4–8 hours, both followed by a 1–48 hour rinse with fresh running water (see Figures 76c and 77c). Both treatments can be applied prior to imbibition, post-imbibition and prior to stratification or post-stratification. Almost all studies have shown these treatments to be effective at reducing *Fusarium* levels on conifer seeds while enhancing cumulative germination. Ching (1960) suggested that enhanced germination may be the result of hydrogen peroxide accelerating respiration.

Technique

In BC, we recommend that post-stratification seed should be immersed in a 3% hydrogen peroxide solution at a 3:1 solution to seed volume ratio for 0.5–4 hours followed by a running water rinse. This recommendation is based

Figure 78 A large single- and a multi-chamber soaking tank used operationally at the BC Ministry of Forests Tree Seed Centre.

Figure 79 Large running water tank used to soak a number of different conifer species seed requests. Tank in the foreground has been left open to show sowing requests; tank in the background is covered to ensure uniform imbibition.

on previous studies and the following observations: 1) the characteristic "bulking up" of fungi during stratification can be significantly reduced at this time; 2) fully imbibed stratified seeds are less likely to uptake the chemical; 3) a 3% hydrogen peroxide solution is easily obtained and poses substantially less occupational risk to workers; and 4) the treatment can be carried out at any facility with a minimum of equipment just prior to sowing.

The potential for reducing the levels of seedborne *Fusarium* with hydrogen peroxide shows good promise though certain inconsistencies still remain. In particular, *Abies* species do not respond consistently and sometimes unfavorably to hydrogen peroxide treatments. More research and operational studies are needed that address these ongoing issues.

Bleach

Chemical treatment can be deployed to reduce seedborne *Fusarium* inoculum (Campbell and Landis 1990). Sodium hypochlorite or "bleach" has been used for many years as a seed coat sanitation treatment. It is readily available, easy to use and inexpensive at the concentrations generally recommended for seed cleaning. Rates range from 1–5.25% sodium hypochlorite for 2–10 minutes (James et al. 1987; Thomsen and Schmidt 1999; Mittal and Wang 1993; James and Genz 1981; Dumroese et al. 1988; Fraedrich 1996). Most of these treatments were applied prior to stratification. Studies by Trotter (1990) and Axelrood (1990; unpubl.)[6] found inconsistent results with 2.1% sodium hypochlorite for 10 minutes when applied as a post-stratification treatment to Douglas-fir seedlots. As a pre-stratification treatment, other researchers have found this particular treatment to provide excellent reductions in seedborne infestations of *Fusarium* with increases in cumulative germination percentages on a variety of pine species and Douglas-fir seedlots (Dumroese et al. 1988; Wenny and Dumroese 1987). Wenny and Dumroese (1987) stress that this treatment should not be used on seeds of the true firs, larch, and spruces.

...we recommend that seed should be immersed in a 3% hydrogen peroxide solution at a 3:1 solution to seed volume ratio for 0.5–4 hours followed by a running water rinse

Other Techniques

The aforementioned treatments are based on most research efforts to reduce effects of seedborne pathogens on conifer seeds but other techniques have or are being proposed that similarly address this issue. Two of these techniques, ethanol and fungicides, may be detrimental to seed viability and have therefore not been used extensively to reduce seedborne fungi.

Ethanol

Ethanol is commonly found in pathology and medical laboratories to sterilize tools and equipment and is used extensively in the administration of vaccination programs to animals and humans. There are few studies using ethanol as a conifer seed treatment. Typical concentrations/exposure times have been in the 70–75% range for three minutes to 95% for 10 seconds (Dumroese et al. 1988; James et al. 1989a; Trotter 1992, unpubl.[7]). Each of these studies has been on Douglas-fir seedlots and resulted in reduced germination, inconsistent fungal assays across replicates,

6 P.E. Axelrood, unpublished data, 1990, BC Research, Vancouver, BC.

7 D. Trotter, unpublished data, 1992, BC Min. For., Extension Services, Surrey, BC.

Table 10 Summary of hydrogen peroxide treatments to conifer seeds by species

Conifer species	H_2O_2 (%) concentration	Duration (hr)	Timing	Results	Reference
Abies amabilis and *A. grandis*	3, 15, 30	0.5–48	Pre-strat, post-strat	No effects on germination. Poor fungal reductions.	Edwards and Sutherland 1979
Abies lasiocarpa	1, 3	1–16	Post-strat	Variability in germination. Moderate fungal reductions.	Neumann 1997
Larix occidentalis	1, 3	1–16	Post-strat	Increase in germination. Significant fungal reductions.	Neumann 1997
Pinus ponderosa	3	5	Pre-strat	Increase in germination. Significant fungal reductions.	James and Genz 1981
Pseudotsuga menziesii	1	12–48	Pre-strat	Increase in germination.	Ching 1960
Pseudotsuga menziesii	3, 30	0.5–8	Post-strat	Variable germination. Significant fungal reductions.	Neumann 1993
Pseudotsuga menziesii	1, 3	1–16	Post-strat	Increase in germination. Significant fungal reductions.	Neumann 1997
Pseudotsuga menziesii	30	0.67	Pre-strat, post-strat	Significant fungal reductions.	Hoefnagels and Linderman 1999
Pseudotsuga menziesii	3	5	Pre-strat, post-strat	Variable germination. Significant fungal reductions.	Dumroese et al. 1988
Picea abies	30	1	Pre-strat	Moderate fungal reductions.	Motta et al. 1996
Pinus taeda, P. elliottii, P. palustris, P. echyinata	3, 30	4–48 and 0.25–3	Pre-strat	Increase in germination. Significant fungal reductions.	Barnett 1976
Pinus palustris	30	0.92	Pre-strat	Increase in germination. Significant fungal reductions.	Fraedrich 1996

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and observations of physical damage to seed coats. Unlike other treatments, ethanol may be readily imbibed by the seed resulting in tissue damage and lower germination. Therefore, ethanol is not a recommended treatment.

Fungicides

Fungicides have long been used on vegetable and agronomic seedlots to provide protection for general diseases such as seed rot, damping-off, wilt, and root rots. Several active ingredients are combined with other chemical constituents to produce a variety of formulations, like **dusts, wettable powders, emulsifiable concentrates,** and **flowables** (Bennett et al. 1991). The basic requirements for a seed-treatment fungicide are: 1) effectiveness under different climatic conditions; 2) non-phytotoxic; 3) safe to operators and wildlife; 4) leave no harmful residues; 5) compatible with other seed treatments; and 6) low price (Agarwal and Sinclair 1997). It is difficult to find fungicides that have all these traits, particularly when most seed-treatment fungicides are phytotoxic at label rates (Bennett et al. 1991; Thomsen and Schmidt 1999). Commonly used fungicides on conifer seeds are captan, ethazole, and thiram but their negative effects on germination and variable efficacy have reduced their usage (Wenny and Dumroese 1987; Lock and Sutherland 1975; Lamontagne and Wang 1976). As broad-based chemicals they maybe effective against a variety of fungi including beneficial and antagonistic organisms. The use of these pathogen-antagonistic organisms as seed treatments is discussed later.

For *Sirococcus* and *Caloscypha* infected seedlots, a fungicide may be the only viable chemical treatment. Systemic fungicides may be absorbed by seeds and reach internal seedborne fungi (Thomsen and Schmidt 1999). Such fungicides may also provide a level of protection to the developing embryo through early germination phases. In a larger context, the long-term viability of fungicide treatments may be limited because regulatory agencies, environmental groups, worker safety organizations, and governments encourage alternative methods. Additional research in this area is warranted.

Hot Water

A possible seed treatment that may provide a viable alternative to traditional chemicals is hot water. Such treatments have been used widely in agriculture to control seedborne pathogens while maintaining high levels of germination. Treatments typically range from 50–60°C for 5–60 minutes but exact temperatures vary with different seeds and associated pathogen species. (Thomsen and Schmidt 1999; Agarwal and Sinclair 1997). Erdey et al. (1997) found that *Fusarium moniliforme* Sheldon in maize was reduced by 85% with a treatment of hot water for 15 minutes at 55°C. For conifer seeds, James et al. (1988) used microwaves to heat the water and assessed its effects on seedborne fungi of Douglas-fir. Their results indicated a thermal window of 43 and 55.5°C for between 60 and 90 seconds for efficacy on *Fusarium* and *Trichoderma* while maintaining high seed viability. Further work was suggested to determine the response of other conifer species, optimal sowing request size, other fluids (e.g., vegetable oils), and strategies to maintain antagonistic organisms (Dumroese et al. 1988).

Biological Control Micro-organisms

The use of naturally occurring micro-organisms to inhibit the effects of pathogens is a technique gaining popularity, particularly because of problems and restrictions of chemical use. These organisms are selected for their ability to persist long enough on seeds and developing radicles to compete with pathogens and reduce or prevent disease development. Biocontrol organisms used against soilborne pathogens include both bacteria or fungi. *Bacillus, Pseudomonas,* and *Enterobacter* spp. represent some of the bacterial genera currently being investigated for their biocontrol properties. Axelrood et al. (1993) isolated several *Pseudomonas* spp. that significantly reduced *F. oxysporum* in growth room assays on Douglas-fir. Early results suggested that the bacteria may promote seedling survival and root growth. As a seed treatment, *Pseudomonas chlororaphis* reduced the bulking up of *Fusarium* during stratification of Douglas-fir seeds (Hoefnagels and Linderman 1999). Additional study found that the greatest reduction in post-stratification levels of *Fusarium* were achieved when seeds received a pre-stratification treatment of hydrogen peroxide followed by exposure to a solution of live *P. chloroaphis* cells.

...use of naturally occurring micro-organisms to inhibit the effects of pathogens is a technique gaining popularity, because of problems and restrictions of chemical use

Of the fungi used for biocontrol of soilborne pathogens, various *Trichoderma* spp. have received much attention. *Trichoderma* spp. are commonly found associated with seeds but their role as possible seed coat antagonists is not well understood. *Trichoderma* spp. are saprophytic fungi that can utilize many different food sources including seeds. The effectiveness of *Trichoderma* lies in a combination of competition for nutrients, production of anti-fungal metabolites, and mycoparasitism (Quarles 1993). Non-pathogenic isolates of various *Fusarium* spp. (particularly *F. oxysporum*) may also be used as biocontrol agents. Studies have shown that these isolates can compete with pathogenic strains for nutrients

and infection sites while conferring some enhanced resistance in the host (Mandeel 1996). Numerous non-pathogenic *Fusarium* isolates have been found on Douglas-fir seedlings (Axelrood et al. 1995; James et al. 1989b) suggesting the possibility that these isolates may confer some level of protection to conifer seeds and seedlings.

For biocontrol organisms to be successful commercial seed treatments, they must meet a variety of conditions: 1) they must be harmless to the seeds, seedling roots, and people; 2) they must demonstrate efficacy under different environmental conditions; 3) they should be active during germination and then be able to colonize developing plants roots; 4) appropriate fungal structures need to be available at high levels; and 5) these structures must withstand drying and storage (Jensen 1996; Taylor and Harman 1990). Improved delivery systems (e.g., pelleting, film coats) for these biocontrol agents are needed (Taylor et al. 1998).

Seed Equipment Sanitation

Background

The elimination or reduction of initial inoculum sources is one component of a strategy that can help to minimize subsequent losses due to seedborne pathogens (Berger 1977). Seed handling has been identified as one of the prime avenues of seed contamination (Littke 1996). Mittal and Wang (1993) found that the incidence of fungi on pine and spruce seeds was low at cone harvest, but increased during air drying of cones as well as the cone and seed processing operations. It is paramount that only clean equipment and containers be used to avoid possible cross contamination among seedlots (Thomsen and Schmidt 1999).

Cone and seed processing and sowing procedures offer many opportunities where fungi may infest seeds

Cone and seed processing and sowing procedures offer many opportunities where fungi may infest seeds. Not only may seeds be brought into contact with contaminated equipment but also clean seeds may be in close physical contact with contaminated seeds.

Techniques

In BC, all processing equipment and areas are cleaned between seedlot batches using vacuuming, sweeping, and air hosing methods. Liquid separation tanks are rinsed with water and wiped. A preliminary study reviewing each stage of the seed extraction process at the BC Ministry of Forests Tree Seed Centre found little or no *Fusarium* on the final seed product of a single seedlot (Neumann 1993). It was noted that the presence of *Fusarium* in other seedlots warranted a larger survey of the seed processing phase. In a follow-up study on seed preparation for stratification, Neumann (1996) found that pathogen inoculum from the ambient air and drying room screens were very low, but that the running water soak tanks and soaking screens used for imbibition had significant *Fusarium* inoculum. Based on subsequent trials, Neumann (1997) recommended that seed soaking tanks should be cleaned using an Ivory® dishwashing soap and hot water scrub treatment during the seed preparation season and that the tank bottoms in particular should be cleaned and rinsed thoroughly. In addition, welding points were shown to habour high *Fusarium* levels. Limiting the size and number of welds will limit the crevices in which fungi can reside Optimally, the cleaning protocol should be carried out weekly but bi-weekly cleanings are adequate to prevent the build-up of *Fusarium* inoculum. An alternative is to fill the tanks with a 3% hydrogen peroxide solution with sufficient volume to soak the bottom of the tanks. The seed soaking screens should be cleaned bi-weekly using a 3–4 hour soak in a 0.5% bleach and buffer solution followed by a 30 minute water rinse.

Overall, sanitation should be an integral part of every step in the seed handling process. All work surfaces should be cleaned and wiped with domestic cleaners or low concentration sterilants (e.g., 0.5% bleach solution). In addition, debris must be discarded from work areas and all tools and instruments that are used directly on seeds disinfected before and after use (Thomsen and Schmidt 1999).

Many commercially important tree species used in reforestation can be grown from seeds, and because maintenance of genetic diversity is so important in ecosystem management, seed propagation is encouraged wherever possible (Landis et al. 1998). However, profitability in forest seedling production depends on the ability to produce a high proportion of the crop within target sizes. To accomplish this one strives to reduce variability.

Propagation by seeds ensures that genetic diversity is maintained by allowing genetic recombination to occur through sexual reproduction. It also lends itself well to mechanization (Figure 80), allowing substantial gains in the accuracy and speed of seed placement and efficiency of seed use. Crops sown quickly aid in initiation of crop uniformity, allowing optimization of inputs later in the crop cycle. This holds true for seeds sown in growing media in containers as well as in soil in an open field. Generally, when seeds are sown in containers, it is easier to provide optimum, uniform, and controlled growing conditions, leading to greater seed use efficiency. In a field situation, weather, soil, competing organisms, and plant spacing are less controllable, increasing variability in growing conditions and lowering seed use efficiency.

Propagation by seeds ensures that genetic diversity is maintained by allowing genetic recombination to occur through sexual reproduction

Seeds are normally sown after stratification and prior to radicle emergence. If germination capacity (GC) or vigour is below desired levels, it may be possible to upgrade seed quality by separating non-viable or less vigorous seeds from viable and/or more vigorous seeds (see "Cone and Seed Processing" chapter for discussion on seed upgrading).

In containers it is imperative that virtually every cavity produces a seedling. With the trend to bigger cavities this is especially true since the investment of nursery inputs per cavity is also larger, although the cost of seed forms a smaller proportion of the final seedling production cost. If non-viable seeds are not separated out, a nursery generally increases the **sowing factor (SF)** or number of seeds sown per cavity (Figure 81) so that the probability of obtaining a viable seedling in each cavity is closer to 100%. Given the GC of a seedlot, the sowing factor can be determined using appropriate probability equations (Schwartz 1993).

Even when a viable seedling is growing in every cavity, not all will meet contract specifications at the end of the production cycle. To account for non-productive cavities (Figure 82), extras are sown. The ratio of extra cavities sown is termed the correction or **oversow factor (OF)** and is expressed as a percent. It varies with grower expertise relative to insect and disease pressures, seedlot characteristics, space, available container type, and contract specifications required by the seedling buyer. If seedling specifications are challenging the nursery may choose to sow a higher number of extra cavities from which to select the best trees.

Seeding equipment is not 100% accurate or efficient. Some seeds are lost during operation and sowing equipment has a minimum requirement for seeds just to allow complete and accurate sowing of a single block, in particular the last few blocks of a request. This means some seeds are continually lost and some will be left over. To facilitate this a small amount of extra seeds, termed the nursery-handling factor, is normally allocated (Figure 83).

Taking all the factors into account, the actual number of seeds supplied per seedling is calculated as follows: (sowing factor × oversow factor) + (nursery-handling factor × oversow factor). Multiplying the seeds supplied per seedling by the GC of a seedlot yields the number of viable seeds supplied per seedling. The number of viable seeds is valuable if contemplating a change in sowing procedures and/or seed upgrading at the nursery.

Sowing Guidelines

Seed returns, simulations and nursery surveys form the basis for average seed use. From this information sowing guidelines are formulated and included in the Seed Planning and Registry system (SPAR) to calculate grams of seeds required for the growth of a specific quantity of seedlings. At the nursery these facilitate the most efficient use of seeds in the production of forest seedlings. The guidelines are designed to create a consistent relationship between GC and the number of seeds supplied per requested seedling (compare 1999, 2001 guidelines with 1996 in Figure 84). The Ministry of Forests guidelines are the SPAR default, but these can be overwritten by the client, preferably in consultation with the nursery.

The flatter curve and more consistent relationship represented in the 1999 and 2001 lines (Figure 84) required the use of more accurate input variables (i.e., GC ranges of 2%, fractional

Seed Sowing Flow Chart (containers)

Process	Objectives
Growing media preparation	Provide uniform mixing and maintenance of structural integrity
Container filling	Insert equal volumes of media into each cavity at a uniform predetermined density
Clean and tamp individual cavities	Provide a firm seedbed and a space at the top of each cavity for the placement of a seed cover (grit)
Seed sowing	Place the prescribed number of seeds in the centre of each growing cavity
Gritting	Place a uniform depth of seed cover on each cavity such that seed is not visible but mechanical impediment to germination is minimized
Seedlot tracking	Seedlot identification on Styroblock® containers
Sowing line watering	Maintain seed imbibition until irrigation system in the growing facility can take over
Transport and layout in growing area	Maintain central seed placement and seed cover integrity
Overall	**Strive to achieve the above as quickly as possible to facilitate uniform germination**

Figure 80
Flow chart of activities in a mechanized seed sowing system.

Figure 81 Multiple sown cavities prior to thinning.

Figure 83 Loaded cams on a cam-drop seeder illustrating the requirement for a nursery-handling factor.

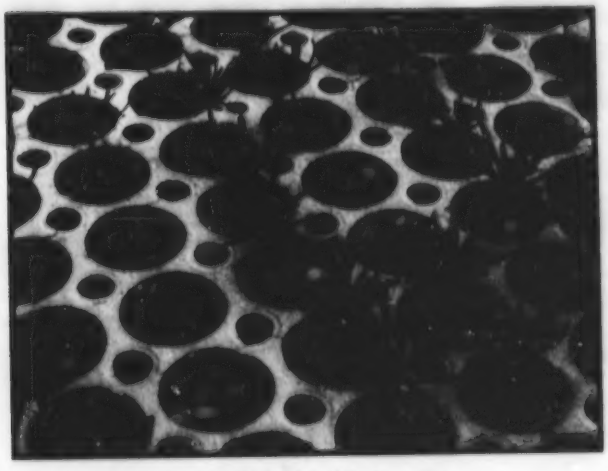

Figure 82 Empty, "non-productive" cavities in a crop.

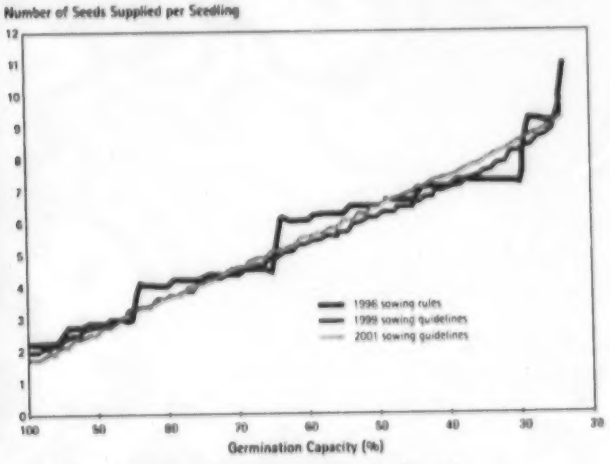

Figure 84 Comparison between 1996 sowing rules and 1999 and 2001 sowing guidelines.

sowing factor increments of 0.1 seeds per cavity, and OF intervals of 1%). Adjustments in 2001 have included a decrease in seed allocated to seedlots with GC values greater than 88% and an increase in seed allocation for seedlots below 80% GC (Figure 84).

Using probability equations, sowing factors (number of seeds supplied per growing cavity) are adjusted for each GC range to minimize the number of empty cavities generated. Taking 92% germination as an example (Table 11), it follows that sowing 1.8 seeds per cavity generates empty cavities with a probability of 2.11%. The oversow factor (suggested extra cavities to sow over and above request) is subsequently adjusted to achieve "cavities with a green tree count" of 125% of the number of seedlings requested. In this case, sowing 1.28 or 28% extra cavities is required to yield a 125% "green tree" count. This means the nursery produces 25% extra trees over and above the requested amount from which to select those seedlings which will be shipped to the customer. In addition, 0.2 extra seeds are supplied per cavity sown ("nursery-handling factor") to allow for sowing equipment minimum operating requirements and inefficiencies.

Following the guidelines in conjunction with the application of appropriate growing techniques and seedling specifications should allow the selection of 100% of requested seedlings. However, if between the nursery and the seed owner a different formula for seed deployment is desired, this can be instituted. The Ministry of Forests sowing guidelines is the default used by SPAR.

If nurseries wish to upgrade sowing requests or change sowing and/or correction factors to suit individual preferences, information on "seeds supplied per seedling requested" is available (Table 11). For example, a seedling request comes with 3000 grams of seeds at 72% GC that after upgrading yield 2000 grams of seeds at 92%. Using the guidelines at 72% GC, we know that 4.58 seeds will be allocated per requested seedling (3.28 viable seeds per seedling). To obtain a 125% green tree count the guidelines

Table 11 Factors and seeds per seedling by germination capacity (%)

Germ %	Sowing factor	Empty cavities (%)	Desired green stem count (%)	OF # of extra cavities to sow	NHF extra # seeds/ cavity sown	Total seeds/ seedling 1996	Total seeds/ seedling 1999	Total seeds/ seedling 2001	Viable seeds/ seedling 2001
100	1.2	0.00	125	1.26	0.20	2.25	2.00	1.76	1.75
99	1.2	0.80	125	1.26	0.20	2.25	2.00	1.76	1.75
98	1.3	1.41	125	1.28	0.20	2.25	2.13	1.92	1.87
97	1.3	2.13	125	1.28	0.20	2.25	2.13	1.92	1.86
96	1.5	2.08	125	1.28	0.20	2.25	2.39	2.18	2.09
95	1.5	2.63	125	1.28	0.20	2.78	2.39	2.18	2.07
94	1.7	2.05	125	1.28	0.20	2.78	2.52	2.43	2.29
93	1.7	2.44	125	1.28	0.20	2.78	2.52	2.43	2.26
92	1.8	2.11	125	1.28	0.20	2.89	2.77	2.56	2.36
91	1.8	2.45	125	1.28	0.20	2.89	2.77	2.56	2.33
90	2	1.00	125	1.27	0.20	2.89	2.90	2.78	2.49
89	2	1.21	125	1.27	0.20	3.00	2.90	2.78	2.49
88	2.2	1.19	125	1.27	0.20	3.00	3.03	3.04	2.68
87	2.2	1.40	125	1.27	0.20	3.00	3.03	3.04	2.65
86	2.4	1.29	125	1.27	0.20	3.00	3.28	3.30	2.84
85	2.4	1.49	125	1.27	0.20	4.09	3.28	3.30	2.81
84	2.5	1.48	125	1.27	0.20	4.09	3.41	3.43	2.88
83	2.5	1.69	125	1.27	0.20	4.09	3.41	3.43	2.85
82	2.7	1.38	125	1.27	0.20	4.09	3.66	3.68	3.02
81	2.7	1.56	125	1.27	0.20	4.09	3.66	3.68	2.98
80	2.8	1.44	125	1.27	0.20	4.25	3.79	3.81	3.05
79	2.8	1.62	125	1.27	0.20	4.25	3.79	3.81	3.01
78	3	1.06	125	1.27	0.20	4.25	3.92	4.05	3.14
77	3	1.22	125	1.27	0.20	4.25	3.92	4.05	3.13
76	3.1	1.28	125	1.27	0.20	4.25	4.18	4.19	3.19
75	3.1	1.45	125	1.27	0.20	4.41	4.18	4.19	3.14
74	3.2	1.50	125	1.27	0.20	4.41	4.31	4.32	3.20
73	3.2	1.68	125	1.27	0.20	4.41	4.31	4.32	3.15
72	3.4	1.56	125	1.27	0.20	4.41	4.44	4.58	3.29
71	3.4	1.75	125	1.27	0.20	4.41	4.44	4.58	3.25
70	3.6	1.57	125	1.27	0.20	4.58	4.58	4.83	3.38
69	3.6	1.75	125	1.27	0.20	4.58	4.58	4.83	3.33
68	3.7	1.72	125	1.27	0.20	4.58	4.71	4.97	3.37
67	3.7	1.91	125	1.27	0.20	4.58	4.71	4.97	3.32
66	3.9	1.60	125	1.27	0.20	4.58	4.97	5.22	3.44
65	3.9	1.78	125	1.27	0.20	6.15	4.97	5.22	3.38
64	4	1.68	125	1.27	0.20	6.15	5.11	5.35	3.41
63	4	1.87	125	1.27	0.20	6.15	5.11	5.35	3.36
62	4.2	1.83	125	1.28	0.20	6.15	5.36	5.61	3.46
61	4.2	2.03	125	1.28	0.20	6.15	5.36	5.61	3.44
60	4.3	2.10	125	1.28	0.20	6.36	5.50	5.76	3.46
59	4.3	2.33	125	1.28	0.20	6.36	5.50	5.76	3.40
58	4.4	2.39	125	1.28	0.20	6.36	5.65	5.90	3.42
57	4.4	2.64	125	1.28	0.20	6.36	5.65	5.90	3.36
56	4.6	2.49	125	1.29	0.20	6.36	5.79	6.16	3.44
55	4.6	2.75	125	1.29	0.20	6.57	5.79	6.16	3.41
54	4.7	2.78	125	1.29	0.20	6.57	6.06	6.31	3.41
53	4.7	3.07	125	1.29	0.20	6.57	6.06	6.31	3.35
52	4.9	2.82	125	1.29	0.20	6.57	6.21	6.57	3.42
51	4.9	3.12	125	1.29	0.20	6.57	6.21	6.57	3.36
50	5	3.13	125	1.29	0.20	6.78	6.37	6.72	3.35
49	5	3.45	125	1.29	0.20	6.78	6.37	6.72	3.29
48	5.1	3.62	125	1.30	0.20	6.78	6.53	6.88	3.31
47	5.1	3.99	125	1.30	0.20	6.78	6.53	6.88	3.24
46	5.3	3.96	125	1.31	0.20	6.78	6.70	7.18	3.29
45	5.3	4.35	125	1.31	0.20	7.21	6.70	7.18	3.24
44	5.4	4.54	125	1.32	0.20	7.21	6.88	7.35	3.23
43	5.4	4.98	125	1.32	0.20	7.21	6.88	7.35	3.18
42	5.5	5.19	125	1.33	0.20	7.21	7.06	7.53	3.16
41	5.5	5.68	125	1.33	0.20	7.21	7.06	7.53	3.11
40	5.6	5.91	125	1.34	0.20	7.42	7.26	7.73	3.09
39	5.6	6.47	125	1.34	0.20	7.42	7.26	7.73	3.03
38	5.7	6.72	125	1.35	0.20	7.42	7.47	7.94	3.00
37	5.7	7.35	125	1.35	0.20	7.42	7.47	7.94	2.95
36	5.8	7.65	125	1.36	0.20	7.42	7.69	8.15	2.92
35	5.8	8.35	125	1.36	0.20	7.42	7.69	8.15	2.86
34	5.9	8.69	125	1.38	0.20	7.42	7.93	8.38	2.84
33	5.9	9.49	125	1.38	0.20	7.42	7.93	8.38	2.78
32	6	9.89	125	1.40	0.20	7.42	8.20	8.65	2.76
31	6	10.79	125	1.40	0.20	7.42	8.20	8.65	2.69
30	6	11.76	125	1.42	0.20	9.28	8.49	8.84	2.64
29	6	12.81	125	1.42	0.20	9.28	8.49	8.84	2.57
28	6	13.93	125	1.46	0.20	9.28	8.81	9.06	2.52
27	6	15.13	125	1.46	0.20	9.28	8.81	9.06	2.46
26	6	16.42	125	1.51	0.20	9.28	9.18	9.35	2.42
25	6	17.80	125	1.51	0.20	11.13	10.00	9.35	2.36

suggest these seeds be sown at 3.4 seeds per cavity at 1.27 oversow (carrying 1.56% empties). After upgrading we are left with 2/3 of the seed remaining or 4.88 × 2/3 = 3.05 seeds supplied per seedling (3.05 × 0.92 = 2.8 viable seeds per seedling). This is more seeds than would have been allocated by SPAR had the seedlot been 92% GC to begin with (2.56 total seeds). Hence to obtain a 125% green stem count the latter can be sown at 1.8 seeds per cavity, 1.28 oversow (carrying 2.11% empties) with some seeds left over. By removing damaged, diseased, or low vigour seeds, the upgraded seeds may display more vigour, resulting in increased germination speed and subsequent improvements in crop uniformity and health.

The nursery has a huge range of sowing strategies at its disposal. For the original 72% seedlot one can choose to single sow at a correction factor up to 3.82 giving a green stem count of 275%. However, to obtain a more reasonable green stem count of 125%, a correction factor of 1.74 is all that is required if single sowing each cavity (note that single-seed sowing results in carrying 28% empty cavities). Moving to the other end of the scale one could sow 4 seeds per cavity at a correction factor of 1.09 since that is as far as the seeds supplied will "stretch." The proportion of empty cavities is minimal at 0.61% but the limited green stem count may increase the difficulty of making request numbers if cull rates are high.

Note from the "seed use" column in Table 12 that certain strategies result in substantial quantities of seeds left over compared to seeds supplied per seedling.

For a given number of seeds supplied per seedling, the combination of sowing and correction factor chosen is up to the grower. There may be situations where a nursery is willing to accept a higher proportion of empties in order to have a larger oversow from which to select final crop trees or generate overruns. Accomplished growers might wish to reduce the oversow factor in favour of increasing the sowing factor, thereby reducing the percent empties and total space allotted to growing an individual request. The latter scenario leaves growing area available for the production of request seedlings under other contracts.

Negotiating seed requirements between the seed owner and nursery can result in increased seed use efficiency

Sowing Guideline Calculations

Use Table 11 to determine the number of seeds per seedling based on the GC of a seedlot. The GC and seeds per gram for all seedlots are available from SPAR. Next, use the following formula to calculate 'grams of seeds' required for a seedling request. Based on the Ministry of Forests *Sowing Guidelines* (2001):

$$\text{Grams of seeds} = \frac{\text{\# of seedlings requested} \times \text{Seeds supplied per seedling}}{\text{Seeds per gram}}$$

Alternatively the number of potential seedlings from a given quantity of seeds can be calculated by rearranging the equation as shown below. The trees per gram available on SPAR is the total potential seedlings divided by the total grams in a seedlot.

$$\text{Potential seedlings} = \frac{\text{Grams of seeds} \times \text{Seeds per gram}}{\text{Seeds supplied per seedling}}$$

Table 12 Some seed-use scenarios at 72 and 92% germination capacity.

Germination capacity (%)	Seeds per seedling		SF*	OF	% empties	GSC %	Seed use
72	4.58	(from Table 11)	3.4	1.27	1.56	125	4.58
		option	4	1.09	0.61	108	4.58
		option	3	1.28	2.2	125	4.10
		option	2	1.36	7.8	125	2.99
		option	1	1.74	28.0	125	2.09
			1	3.82	28.0	275	4.58
92	2.96	(from Table 11)	1.8	1.28	2.11	125	2.56
		option	1.5	1.31	4.32	125	2.23
		option	1	1.36	8.0	125	1.63
			1	2.13	8.0	196	2.56

Seed use = (SF*OF) + (0.2*OF)
% empties = $(1\text{-GC}/100)^{SF}$
Green stem count = OF – (% empties*OF)

*SF = sowing factor
OF = oversow factor
GSC = greenstem count

Table 11 Factors and seeds per seedling by germination capacity (%)

Germ %	Sowing factor	Empty cavities (%)	Desired green stem count (%)	OF # of extra cavities to sow	NHF extra # seeds/ cavity sown	Total seeds/ seedling 1996	Total seeds/ seedling 1999	Total seeds/ seedling 2001	Viable seeds/ seedling 2001
100	1.2	0.00	125	1.26	0.20	2.25	2.00	1.76	1.75
99	1.2	0.80	125	1.26	0.20	2.25	2.00	1.76	1.75
98	1.3	1.41	125	1.28	0.20	2.25	2.13	1.92	1.87
97	1.3	2.13	125	1.28	0.20	2.25	2.13	1.92	1.86
96	1.5	2.08	125	1.28	0.20	2.25	2.39	2.18	2.09
95	1.5	2.63	125	1.28	0.20	2.78	2.39	2.18	2.07
94	1.7	2.05	125	1.28	0.20	2.78	2.52	2.43	2.29
93	1.7	2.44	125	1.28	0.20	2.78	2.52	2.43	2.26
92	1.8	2.11	125	1.28	0.20	2.89	2.77	2.56	2.36
91	1.8	2.45	125	1.28	0.20	2.89	2.77	2.56	2.33
90	2	1.00	125	1.27	0.20	2.89	2.90	2.78	2.49
89	2	1.21	125	1.27	0.20	3.00	2.90	2.78	2.49
88	2.2	1.19	125	1.27	0.20	3.00	3.03	3.04	2.68
87	2.2	1.40	125	1.27	0.20	3.00	3.03	3.04	2.65
86	2.4	1.29	125	1.27	0.20	3.00	3.28	3.30	2.84
85	2.4	1.49	125	1.27	0.20	4.09	3.28	3.30	2.81
84	2.5	1.48	125	1.27	0.20	4.09	3.41	3.43	2.88
83	2.5	1.69	125	1.27	0.20	4.09	3.41	3.43	2.85
82	2.7	1.38	125	1.27	0.20	4.09	3.66	3.68	3.02
81	2.7	1.56	125	1.27	0.20	4.09	3.66	3.68	2.98
80	2.8	1.44	125	1.27	0.20	4.25	3.79	3.81	3.05
79	2.8	1.62	125	1.27	0.20	4.25	3.79	3.81	3.01
78	3	1.06	125	1.27	0.20	4.25	3.92	4.05	3.14
77	3	1.22	125	1.27	0.20	4.25	3.92	4.05	3.13
76	3.1	1.28	125	1.27	0.20	4.25	4.18	4.19	3.19
75	3.1	1.45	125	1.27	0.20	4.41	4.18	4.19	3.14
74	3.2	1.50	125	1.27	0.20	4.41	4.31	4.32	3.20
73	3.2	1.68	125	1.27	0.20	4.41	4.31	4.32	3.15
72	3.4	1.56	125	1.27	0.20	4.41	4.44	4.58	3.29
71	3.4	1.75	125	1.27	0.20	4.41	4.44	4.58	3.25
70	3.6	1.57	125	1.27	0.20	4.58	4.58	4.83	3.38
69	3.6	1.75	125	1.27	0.20	4.58	4.58	4.83	3.33
68	3.7	1.72	125	1.27	0.20	4.58	4.71	4.97	3.37
67	3.7	1.91	125	1.27	0.20	4.58	4.71	4.97	3.32
66	3.9	1.60	125	1.27	0.20	4.58	4.97	5.22	3.44
65	3.9	1.78	125	1.27	0.20	6.15	4.97	5.22	3.38
64	4	1.68	125	1.27	0.20	6.15	5.11	5.35	3.41
63	4	1.87	125	1.27	0.20	6.15	5.11	5.35	3.36
62	4.2	1.83	125	1.28	0.20	6.15	5.36	5.61	3.46
61	4.2	2.03	125	1.28	0.20	6.15	5.36	5.61	3.44
60	4.3	2.10	125	1.28	0.20	6.36	5.50	5.76	3.46
59	4.3	2.33	125	1.28	0.20	6.36	5.50	5.76	3.40
58	4.4	2.39	125	1.28	0.20	6.36	5.65	5.90	3.42
57	4.4	2.64	125	1.28	0.20	6.36	5.65	5.90	3.36
56	4.6	2.49	125	1.29	0.20	6.36	5.79	6.16	3.44
55	4.6	2.75	125	1.29	0.20	6.57	5.79	6.16	3.41
54	4.7	2.78	125	1.29	0.20	6.57	6.06	6.31	3.41
53	4.7	3.07	125	1.29	0.20	6.57	6.06	6.31	3.35
52	4.9	2.82	125	1.29	0.20	6.57	6.21	6.57	3.42
51	4.9	3.12	125	1.29	0.20	6.57	6.21	6.57	3.36
50	5	3.13	125	1.29	0.20	6.78	6.37	6.72	3.35
49	5	3.45	125	1.29	0.20	6.78	6.37	6.72	3.29
48	5.1	3.62	125	1.30	0.20	6.78	6.53	6.88	3.31
47	5.1	3.99	125	1.30	0.20	6.78	6.53	6.88	3.24
46	5.3	3.96	125	1.31	0.20	6.78	6.70	7.18	3.29
45	5.3	4.35	125	1.31	0.20	7.21	6.70	7.18	3.24
44	5.4	4.54	125	1.32	0.20	7.21	6.88	7.35	3.23
43	5.4	4.98	125	1.32	0.20	7.21	6.88	7.35	3.18
42	5.5	5.19	125	1.33	0.20	7.21	7.06	7.53	3.16
41	5.5	5.68	125	1.33	0.20	7.21	7.06	7.53	3.11
40	5.6	5.91	125	1.34	0.20	7.42	7.26	7.73	3.09
39	5.6	6.47	125	1.34	0.20	7.42	7.26	7.73	3.03
38	5.7	6.72	125	1.35	0.20	7.42	7.47	7.94	3.00
37	5.7	7.35	125	1.35	0.20	7.42	7.47	7.94	2.95
36	5.8	7.65	125	1.36	0.20	7.42	7.69	8.15	2.92
35	5.8	8.35	125	1.36	0.20	7.42	7.69	8.15	2.86
34	5.9	8.69	125	1.38	0.20	7.42	7.93	8.38	2.84
33	5.9	9.49	125	1.38	0.20	7.42	7.93	8.38	2.78
32	6	9.89	125	1.40	0.20	7.42	8.20	8.65	2.76
31	6	10.79	125	1.40	0.20	7.42	8.20	8.65	2.69
30	6	11.76	125	1.42	0.20	9.28	8.49	8.84	2.64
29	6	12.81	125	1.42	0.20	9.28	8.49	8.84	2.57
28	6	13.93	125	1.46	0.20	9.28	8.81	9.06	2.52
27	6	15.13	125	1.46	0.20	9.28	8.81	9.06	2.46
26	6	16.42	125	1.51	0.20	9.28	9.18	9.35	2.42
25	6	17.80	125	1.51	0.20	11.13	10.00	9.35	2.36

suggest these seeds be sown at 3.4 seeds per cavity at 1.27 oversow (carrying 1.56% empties). After upgrading we are left with 2/3 of the seed remaining or 4.88 × 2/3 = 3.05 seeds supplied per seedling (3.05 × 0.92 = 2.8 viable seeds per seedling). This is more seeds than would have been allocated by SPAR had the seedlot been 92% GC to begin with (2.56 total seeds). Hence to obtain a 125% green stem count the latter can be sown at 1.8 seeds per cavity, 1.28 oversow (carrying 2.11% empties) with some seeds left over. By removing damaged, diseased, or low vigour seeds, the upgraded seeds may display more vigour, resulting in increased germination speed and subsequent improvements in crop uniformity and health.

The nursery has a huge range of sowing strategies at its disposal. For the original 72% seedlot one can choose to single sow at a correction factor up to 3.82 giving a green stem count of 275%. However, to obtain a more reasonable green stem count of 125%, a correction factor of 1.74 is all that is required if single sowing each cavity (note that single-seed sowing results in carrying 28% empty cavities). Moving to the other end of the scale one could sow 4 seeds per cavity at a correction factor of 1.09 since that is as far as the seeds supplied will "stretch." The proportion of empty cavities is minimal at 0.61% but the limited green stem count may increase the difficulty of making request numbers if cull rates are high.

Note from the "seed use" column in Table 12 that certain strategies result in substantial quantities of seeds left over compared to seeds supplied per seedling.

For a given number of seeds supplied per seedling, the combination of sowing and correction factor chosen is up to the grower. There may be situations where a nursery is willing to accept a higher proportion of empties in order to have a larger oversow from which to select final crop trees or generate overruns. Accomplished growers might wish to reduce the oversow factor in favour of increasing the sowing factor, thereby reducing the percent empties and total space allotted to growing an individual request. The latter scenario leaves growing area available for the production of request seedlings under other contracts.

Negotiating seed requirements between the seed owner and nursery can result in increased seed use efficiency

Sowing Guideline Calculations

Use Table 11 to determine the number of seeds per seedling based on the GC of a seedlot. The GC and seeds per gram for all seedlots are available from SPAR. Next, use the following formula to calculate 'grams of seeds' required for a seedling request. Based on the Ministry of Forests *Sowing Guidelines* (2001):

$$\text{Grams of seeds} = \frac{\text{\# of seedlings requested} \times \text{Seeds supplied per seedling}}{\text{Seeds per gram}}$$

Alternatively the number of potential seedlings from a given quantity of seeds can be calculated by rearranging the equation as shown below. The trees per gram available on SPAR is the total potential seedlings divided by the total grams in a seedlot.

$$\text{Potential seedlings} = \frac{\text{Grams of seeds} \times \text{Seeds per gram}}{\text{Seeds supplied per seedling}}$$

Table 12 Some seed-use scenarios at 72 and 92% germination capacity.

Germination capacity (%)	Seeds per seedling		SF*	OF	% empties	GSC %	Seed use
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		option	3	1.28	2.2	125	4.10
		option	2	1.36	7.8	125	2.99
		option	1	1.74	28.0	125	2.09
			1	3.82	28.0	275	4.58
92	2.96	(from Table 11)	1.8	1.28	2.11	125	2.56
		option	1.5	1.31	4.32	125	2.23
		option	1	1.36	8.0	125	1.63
			1	2.13	8.0	196	2.56

Seed use = (SF*OF) + (0.2*OF)
% empties = $(1-GC/100)^{SF}$
Green stem count = OF – (% empties*OF)

*SF = sowing factor
OF = oversow factor
GSC = greenstem count

Sample calculation

Number of seedlings requested = 15 200
Seedlot # 60277, GC = 91%, Seeds per gram = 349

$$\text{Grams required} = \frac{15\,200 \text{ seedlings} \times 2.56 \text{ seeds supplied per seedling}}{349 \text{ seeds per gram}} = 111.49$$

Note: Seed withdrawals at the Tree Seed Centre are carried out to the nearest gram (whole number). SPAR automatically rounds the calculation upward to the nearest whole gram. You may see the message *'Potential trees have been recalculated'* in SPAR when entering a seedling request, due to the rounding factor.

Figure 85 Proper seed storage upon arrival at the nursery.

Practical Sowing Hints/General Guidelines

To help ensure that allotted seeds meet the goals and objectives set out, there are some general guidelines one can follow.

Upon arrival at the nursery seeds should be inspected for mould, colour, moisture status, debris, damage, and deterioration to determine acceptability. If a problem is identified that could compromise the ability of seeds to produce the requested crop, the supplier should be contacted immediately to discuss options. A nursery may choose to address the problem if time and facilities are available. To ensure that enough seeds are available, they should be weighed. (Note that sowing request labels are based on storage moisture content [i.e., dry seed]). If stratified seeds are received, the weight will be appreciably higher due to the moisture imbibed by the seeds. If there are insufficient seeds, the seed owner should be contacted to authorize the release of additional seed. The seedling customer needs to be notified if seedling requests will be compromised. The area where seeds are received, inspected, stratified, and stored should be clean.

Once accepted, it may still be necessary to clean the seeds and re-bag them. If there is suspicion of disease, a sample should be sent to a pathology laboratory. Moisture content should be checked upon arrival and monitored until sowing. This can be carried out using the target moisture content calculations described in the "Seed Pretreatment" chapter (page 53). One needs to know the storage moisture content and the weight of the seeds. With this information you can rapidly evaluate the moisture content of seeds upon receipt, dry them back to a target moisture content if required, and control moisture content during stratification and interim storage. One benefit of using this method to calculate moisture content is that no seeds have to be sacrificed.

Upon arrival at the nursery seeds should be inspected for mould, colour, moisture status, debris, damage, and rot

Large shipments are better split into smaller bags of equal proportions to facilitate handling and maintenance of quality. Bags need to be flattened for efficient storage to maintain uniform moisture content. Bags containing stratified seeds require an opening for gas exchange (Figure 85). Current lab germination and fungal assay results are supplied on the request labels. However, any previous nursery records on a seedlot should be checked to determine its history, other characteristics, and possible problems. The value of keeping your own records cannot be overemphasized. Based on the above, make plans for additional testing, remedial action, and/or performance upgrading.

During sowing, it helps to divide seeds for large requests into halves, thirds, or quarters and monitor their use. If the number of cavities or blocks being sown is also monitored it is easy to determine if seed use is appropriate. For instance, when 50% of allotted seeds are used up the number of cavities sown should total 50% or slightly greater. If half the seeds are consumed to sow only 40% of the required cavities, an adjustment in sowing factor is required for the remaining seeds.

Splitting bags of seeds and keeping only the currently needed seeds out also reduces the risk of seed viability losses through exposure to sub-optimal conditions, which may be present at the sowing line. Heat and drying conditions or exposure to disease inoculum are not uncommon.

Wetting containers (Figure 86) immediately after sowing and seed cover (gritting) application ensures that seed imbibition is not lost or compromised prior to arrival in the growing area (Figure 87) where misting/irrigation systems are in place. The application of moisture at the end of the sowing line is recommended.

Wetting containers immediately after sowing and gritting application ensures that seed imbibition is not lost or compromised prior to arrival in the growing area

Pelletized western redcedar seeds are not imbibed prior to pelleting and wetting containers will mark the beginning of imbibition while on the sowing line. This will require the input of greater quantities of water compared to other species that are already fully imbibed. Once water is applied, the maintenance of imbibition (seed moisture) is especially critical because the applied pellet coating dries easily and can extract moisture from the seeds. Drying also seems to cement the pellet to the germinant, with obvious negative results.

Figure 86 Applying moisture immediately after seeding, prior to transporting containers to the growing area.

Figure 87 Laying out containers in the growing area.

Seeding Equipment

Seeding equipment mechanizes the placement of seeds into growing cavities. The objective is to sow quickly and accurately, gently placing each seed in the centre of its growing cavity. Centring seeds in containers allows seedlings to develop a more symmetrical (balanced) root system (Figure 88). Sowing large crops within short timeframes allows all seedlings to germinate together (Figure 89) thereby establishing a more even-aged crop that responds more uniformly to further management.

Most seeders use vacuum to pick up individual seeds from a common reservoir and transfer them to their respective growing cavities. Vacuum may be applied to small holes from the inside of a drum, back of a flat plate, or hollow needles mounted on a bar or plate (Figure 90). Seeds are released to drop into the growing cavity simply by interrupting the vacuum at the right time, when the orifice holding the seed is positioned precisely over the centre of the growing cavity, and close enough to it so that air currents and vibration will not carry it off course. To ensure that sticky or very light seeds fall off at the appropriate moment, depending on equipment capability a small burst of positive air pressure or a drop of water may be applied in addition to interrupting the vacuum.

The objective is to sow quickly and accurately, gently placing each seed in the centre of its growing cavity

Cam-drop seeders employ a rocking cam to place seeds individually into tubes directed to individual growing cavities (Figure 83). Gentle vibration moves seeds from a common

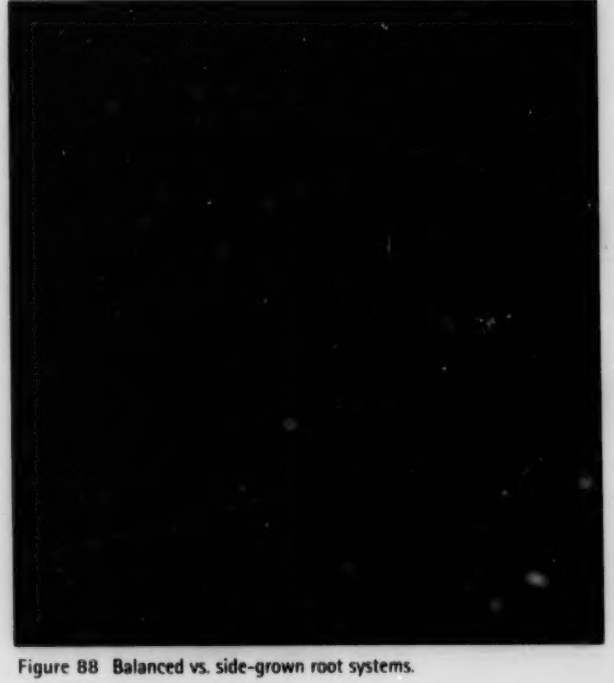

Figure 88 Balanced vs. side-grown root systems.

Figure 89 Uniform germination vs. late germinants (circled).

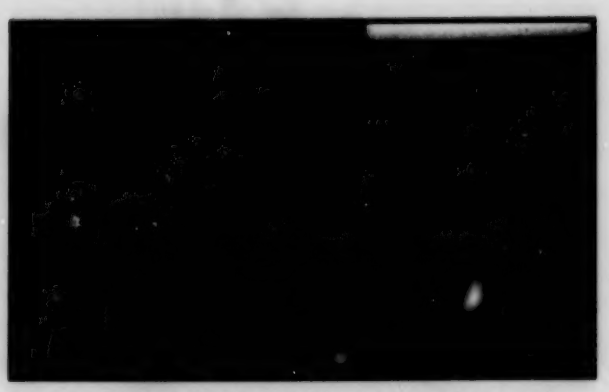

Figure 90 Pelletized seeds being picked up by vacuum from a needle seeder prior to placement.

reservoir into single seed-sized slots cut into the cam. Each "rock" of the cam collects a complement of seeds and drops them into their respective cavities.

Fractional Sowing Strategies

Sowing is performed mechanically to ensure seeds are sown as quickly as possible, thereby establishing maximum crop uniformity.

Fractional sowing means that on average, the number of seeds sown per cavity is not an integer. For example, if the fractional sowing factor is 2.2 seeds per cavity that means that 80% and 20% of the cavities contain two and three seeds respectively. The challenges are how to accurately set up sowing machinery to perform this way and how to distribute the cavities with extra seeds through a crop.

One way to fractionally sow a crop is in two parts. For the above example, 80% and 20% of the cavities are sown at two and three seeds per cavity respectively. All seeding machines available on the market are capable of this (suggested ratios per integer value for sowing factors up to five seeds per cavity are found in Table 13). This method creates two crops within a particular seedling request, each with its own sowing factor. This may make them behave somewhat differently, possibly requiring separate management strategies. However, the strategy may be desirable for sowing factors between one and two seeds/cavity since that portion of the crop sown at one seed per cavity will not require thinning. This method is time consuming if it requires changing or taping seed drums. However, some nurseries possess more than one seeding machine, allowing placement in series. The extra seeding machine merely functions as a conveyor when not being used.

Fractional sowing means that on average, the number of seeds sown per cavity is not an integer

If the fractional sowing factor is greater than two, the whole crop is thinned anyway and distributing cavities with higher integer seeds evenly throughout a crop is desirable. It will make the crop more uniform, simplifying further management. It does, however, require altering the seeder somewhat or changing sowing line procedures. With the vacuum drum seeder a drum with the higher integer value can be utilized and as in our example, with 80% of the extra (third) holes sealed (taped) (Figure 91). The pattern chosen is up to the grower. Linear patterns using tape, or random patterns using small round "stickies," are options. The cam-drop seeder uses small o-rings to interrupt seed placement into selected cavities (Figure 83).

Another way to obtain the same result requires running the crop through the seeder at two seeds per cavity, and then again at 0.2 seeds per cavity. The second time through, the seeder is taped to drop one seed in only 20% of the cavities per block. This is cumbersome but may be an option for some. It can also be done with two seeding machines set up in series. On vacuum drum seeders that employ an airbrush, the operator may use the airbrush to finesse the number of seeds per cavity instead of stopping production to change drums or

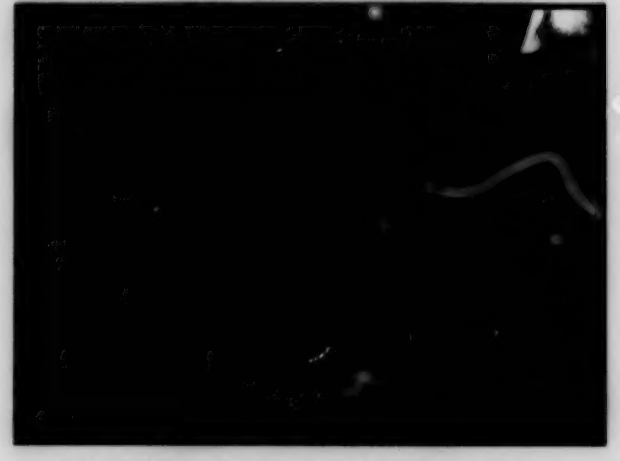

Figure 91 Vacuum seeder drum with proportion of holes taped.

Table 13 Proportion (%) of cavities to sow at a given sowing factor to produce various fractional sowing factors

Fractional sowing factor	Sowing factor (seeds per cavity)				
	1	2	3	4	5
1.0	100				
1.1	90	10			
1.2	80	20			
1.3	70	30			
1.4	60	40			
1.5	50	50			
1.6	40	60			
1.7	30	70			
1.8	20	80			
1.9	10	90			
2.0		100			
2.1		90	10		
2.2		80	20		
2.3		70	30		
2.4		60	40		
2.5		50	50		
2.6		40	60		
2.7		30	70		
2.8		20	80		
2.9		10	90		
3.0			100		
3.1			90	10	
3.2			80	20	
3.3			70	30	
3.4			60	40	
3.5			50	50	
3.6			40	60	
3.7			30	70	
3.8			20	80	
3.9			10	90	
4.0				100	
4.1				90	10
4.2				80	20
4.3				70	30
4.4				60	40
4.5				50	50
4.6				40	60
4.7				30	70
4.8				20	80
4.9				10	90
5.0					100

tape holes. The intended purpose of the airbrush is to increase seeder accuracy by ensuring that only one seed adheres to each hole in the seed drum. However, to increase the number of seeds deposited per cavity above the number of holes in the drum, one can turn down the airbrush thereby promoting multiple seeds adhering to a single hole. Conversely one can utilize a drum with more holes per cavity than required and turn up the airbrush to "blow off" a portion of the seeds. These are very coarse adjustments and require constant monitoring to ensure seed consumption is at the appropriate rate. In the case of high airbrush settings be aware that all holes for certain cavities can easily be blown free of seeds, resulting in the generation of empty cavities.

Vacuum plate and needle seeders can be obtained with plates and needle set-ups containing more than one orifice per cavity to be sown. In this case a three-hole vacuum plate can be taped to 2.2 seeds per cavity. Vacuum needle seeders are available with single, double, and triple needle per cavity designs, which can be mixed on the same plate. Triple needle per cavity designs can be substituted into 20% of an otherwise double needle per cavity plate to achieve the above-stated example.

It is also conceivable to order/manufacture plates and drums with a "fractional" number of holes drilled in them (e.g., 1.5 holes per cavity would mean 50% of the cavities would have two holes drilled in them).

Choosing which Cavities to Multiple-sow

Some cavities by virtue of container design have more growing space at their disposal. Trees supplied with more growing space are generally shorter and produce greater stem diameters than the crop average. The objective is to equally distribute the growing space available per seedling so that overall crop growth is as uniform as possible and photosynthesis per unit growing area is maximized. Depending on the stocktype (species, container size, age, planting window combination) being produced and the type of culls anticipated (based on experience), one chooses which cavities should definitely have a seedling remaining in them after thinning and therefore be preferentially multiple sown. Generally these are the cavities around the perimeter of the block. After they are multiple sown, depending on how many more are needed, one moves toward the centre of the block. Blocks with different numbers of cavities will have different percentages of edge cavities. A 45 cavity block has 24 (53%) edge cavities, a 112 block has 40 (36%) edge cavities, and a 160 block has 48 (30%) edge cavities. If edge cavities in a particular block traditionally yield underheight culls due to excessive drying, one might conclude that these cavities should not be preferentially multiple sown. However, doing this would result in even drier conditions for the remaining edge seedlings, ensuring their demise, as well as effectively moving "edge" conditions further into the centre. It would be better to fill edge cavities and leave some blanks in the centre, and couple this with altered culture to promote more uniform climatic conditions between the centre and perimeter of individual blocks.

Different blocks lend themselves to different patterns. Below are two examples of fractional sowing a 77 cavity block, including achieved fractional sowing rates (Figure 92).

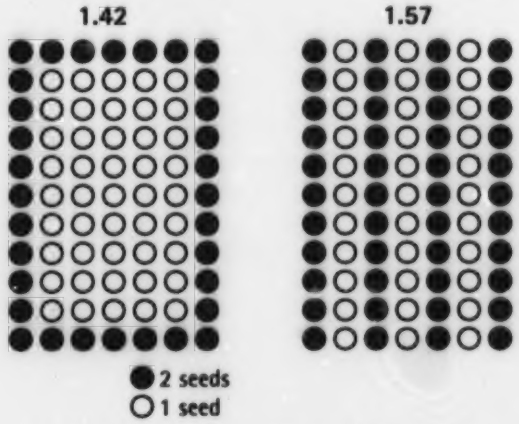

Figure 92 Actual fractional sowing rates through the use of two different configurations for single and double sowing in a 77 cavity block.

Seed germination is the process of emergence of the root and shoot, leading to the growth of a seedling. The ability to choose and implement appropriate cultural practices to achieve this end demands an understanding of the germination requirements of seeds. Seed germination in the nursery can be divided into two stages, where stage one encompasses the time from seed sowing to radicle emergence, or emergence from the growing medium if seeds are covered. Stage two begins with radicle emergence and lasts until the first true leaf emerges. For a summary of stage one and stage two nursery germination guidelines, see Appendix 5.

Stage 1

Metabolic activity begins with the rapid imbibition of water by the cells of the dry seed. Membrane and organelle systems are reorganized and respiration is initiated to release energy from stored reserves for future needs. Ultimately the seed swells and the seed coat splits along the junction of its two halves.

Goal – Germination Stage 1: Quick and even germination, resulting in uniform crop emergence

Enzyme activity begins quickly after the onset of seed hydration. This activity involves previously stored **enzymes** at the very start of hydration but quickly adds currently synthesized enzymes as germination progresses. Basically, the **metabolic** machinery of the cells is "turned on." Reserves of complex molecules are broken down (**hydrolysis**) into smaller constituents. This releases energy, provides building blocks for use in producing new growth, and serves to increase the **osmotic potential** within cells. The latter triggers further uptake of water which serves to expand or telescope the existing embryo.

The first visible evidence of germination is the emergence of the radicle. It results from the elongation of existing cells and usually occurs within a few days, marking the end of stage one.

Goal – Germination Stage 1: Quick and even germination, resulting in uniform crop emergence.

Stage 2

By this time the vascular system has differentiated and become operational. Fats, proteins, and carbohydrates stored in the megagametophyte and embryo are digested to simpler chemical substances and translocated to the growing points of the embryo (root and shoot apical meristems as well as the **vascular cambium**).

Goal – Germination Stage 2: Maintain uniformity, health, and vigour to facilitate quick and predictable responses to future cultural practices

It is during this stage that the "seedling" is forced to switch from relying on rapidly depleting stored energy reserves to producing its own through photosynthesis. Newly exposed green plant parts must begin to **photosynthesize** and the radicle must begin to draw nutrients from the soil solution. Increase in size involves cell division as well as elongation. It is imperative that once "green" is evident, the 24 hour energy balance be managed so that daytime energy accumulation outweighs night-time respiratory losses and maintenance requirements. Growth requires energy accumulation over and above this break-even point. To facilitate this, photosynthetically active radiation of appropriate intensity must be supplied in addition to managing plant temperature to maintain positive net photosynthesis (see Figure 93).

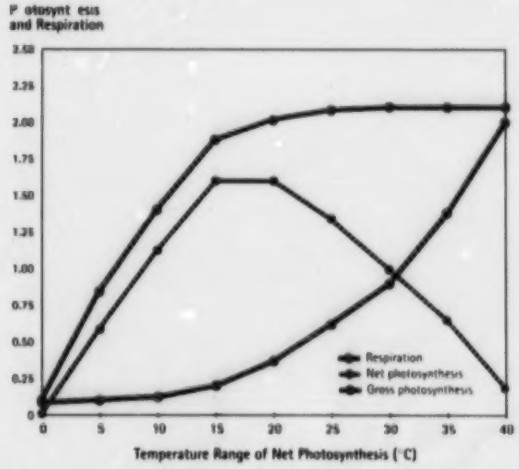

Figure 93 Effects of temperature on photosynthesis, respiration, and net or apparent photosynthesis of Swiss stone pine seedlings (from Tranquiline 1955).

Goal – Germination Stage 2: Maintain uniformity, health, and vigour to facilitate quick and predictable responses to future cultural practices.

Uniformity

Uniformity is a first principle if discussing maximization of forest seedling production efficiency. Growing plants from seeds ensures a certain amount of variability between seeds due to genetic recombination arising from sexual reproduction. In addition, seeds from various parent trees are generally mixed to form a seedlot. Standard (wild) seedlots are normally comprised of seeds from trees of fairly close proximity. Seed orchard seedlots may be comprised of seeds from parents originating from wider geographical areas. The latter can, depending on parent tree selection, be genetically more variable than the former. Using an ingredient with inherent genetic variability (seeds) to produce a product of uniform morphology (seedlings) is a challenge.

Non-uniform or staggered crop emergence results in...lower overall production efficiency

In the nursery the first expression of variability is during germination. To minimize this expression morphologically without losing it genetically, one strives for uniform, rapid crop emergence. This is essential. Non-uniform or staggered crop emergence results in plants that vary in size, nutrient and water uptake requirements, and competitive ability. This reduces the effectiveness of subsequent cultural inputs (nutrients, heat, CO_2, short-day treatment) and lowers overall production efficiency in general. For example, one might delay implementation of short-day treatment on a non-uniform (height) crop to allow short individuals to catch up. Consequently, taller individuals may end up over-height, and budset in the whole crop is induced later with less time devoted to hardening. Sowing earlier to compensate for variability just shifts the cost of inefficiency to the beginning of the crop cycle. Sowing earlier when greenhouse heating costs are higher is an expensive option, shifting peak demands on annual cash flow projections. Starting earlier when light conditions are poorer also increases the risks of contracting disease, possibly requiring a greater oversow factor.

In essence, we are dealing with a "miniature forest," where the degree of variability increases with crop age as size differences compound the variability in competitiveness. Smaller plants receive fewer resources such as light/air movement than larger neighbours causing them to grow at progressively slower rates as they drop relatively lower into the understorey. This can result in substantial proportions of a crop falling outside the specification window, increasing grading costs and culling levels.

Bringing non-uniform crops to market often requires much greater inputs of grower attention, technology, and energy. Using extreme measures to keep a crop going at the beginning can result in excessive growth later on, often requiring more extreme shutdown measures.

The uniformity with which required inputs are supplied governs how well the uniformity established thus far is retained. All seeds need to experience the same temperature, oxygen, moisture, seed cover depth, and light conditions. Inherent differences in germination speed between seeds in a seedlot can be compressed to a certain extent by employing higher germination temperatures (25°C fraction as an example in Figure 94). To increase crop uniformity of the 15°C fraction (Figure 94) one could multiple sow it and thin the late germinants, or raise its germination temperature. If germination speed is high but germination capacity is low (seedlot B in Figure 95), crop germination will be uniform but scattered. In this case one would multiple sow and thin the low germination capacity lot, thereby increasing its percent cavity fill.

Once water touches a seed during the pre-stratification soak, the overall seedling crop cycle essentially begins

Once water touches a seed during the pre-stratification soak, the overall seedling crop cycle essentially begins.

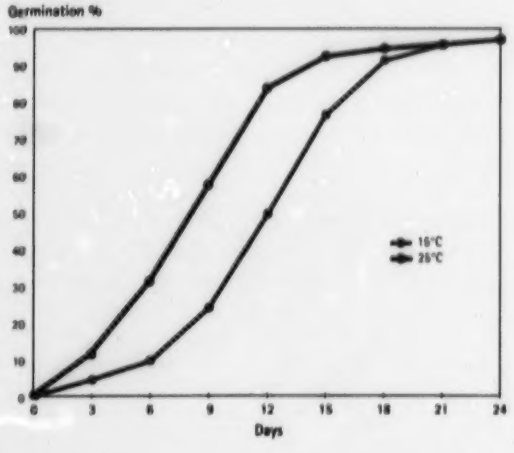

Figure 94 Germination rate comparison of a seedlot at two germination temperatures (25°C and 15°C).

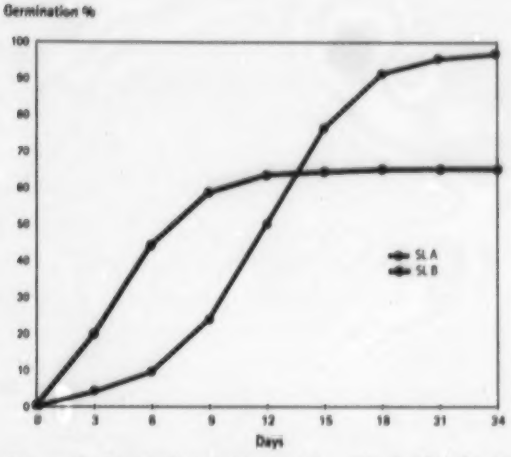

Figure 95 Germination speed vs. total germination (%). Seedlot B will emerge more uniformly even though it yields less viable germinants. Multiple sowing and thinning Seedlot B will increase its percent cavity fill and yield a more uniform crop than single sowing Seedlot A.

Environmental Factors Affecting Germination

Newly sown seeds are placed on top of the growing media, then covered with a thin layer of grit (seed cover). Being in contact with both, seeds are able to lose or gain heat from each of them by conduction. Conventional peat-based growing media and grit are chosen for their high aeration porosity, which can also influence seed moisture content gain or loss. What seeds experience depends on how (forced air, radiant) and from where (above, below) the heat is supplied, and on the moisture conditions of growing media and grit. The argument for under-bench heating generally assumes that seed temperature follows growing media temperature.

It is important to remember that grit is in contact with the seeds and growing media as well. Grit temperature is influenced in large part by above-bench conditions including incoming solar radiation (day), outgoing long wave radiation (night), convection, and irrigation water temperature, for example. Depending upon duration and strength of these influences, the conditions the seeds experience may more closely follow grit conditions. Grit forms part of the surface adjacent to which we find the **laminar boundary layer** (LBL) climate. Depending on depth, particle size distribution, and aeration porosity, some grit types may actually be more appropriately assumed to be within the zone termed the LBL.

Laminar Boundary Layer Climate

Factors that interact to determine the boundary layer climate are:

- light intensity, quality, and duration
- humidity
- temperature of the air, growing media, irrigation water
- plant spacing and size
- canopy density
- air movement
- seed cover heat capacity, aeration porosity, colour, and depth.

The LBL is the layer of air in contact and immediately surrounding a surface, in this case the seeds/germinants as illustrated in Figure 96. It is at most several millimetres to 1 cm in depth. The climate within the LBL influences surface condition most directly. Gross greenhouse or compound environments indirectly assert their influence through alteration of the LBL climate. The thickness of the LBL is governed by the turbulence of adjacent air and surface features of the plant part in question. Horizontal airflow fans in greenhouses reduce the thickness of the boundary layer surrounding needles. Epidermal hairs on needles serve to reduce the influence of horizontal airflow fans, thereby retaining boundary layer thickness and its innate buffering capacity. Grit may be seen to perform a similar function for conifer seeds, which do not have epidermal hairs.

Radiation heats surfaces warming the LBL, evaporation and/or transpiration from the surface increases the humidity of the LBL, and gas exchange through stomata alters its gaseous make-up. Gradients of gases, humidity, and temperature exist from the plant/seed surface to the LBL edge. At the edge the gross climate effects changes in the boundary layer climate mainly through convection (mixing). However, any non-radiative transfer across the LBL is by means of molecular diffusion, which is very slow. Therefore, reducing the thickness of the boundary layer is the quickest way to effect changes at the leaf or seed surface since it increases the steepness of the respective gradients. A thin LBL allows removal of unwanted gases faster. It also allows faster removal of transpired water vapour, allowing a plant to more effectively cool itself. In the case of seeds, a thin LBL coupled with a low humidity gross greenhouse climate can result in accelerated evaporation of surface moisture, leading to cooling and subsequent reduction in germination speed, as well as possible drying. During seed germination a thick boundary layer (calm conditions) coupled with a high humidity gross greenhouse climate is desirable. This prevents possible evaporative cooling and drying of seeds, thereby reducing the need for application of irrigation water, which generally performs a further cooling function. Beyond germination a more active climate (lower humidity, horizontal airflow) is more beneficial.

The laminar boundary layer is the layer of air in contact and immediately surrounding a surface

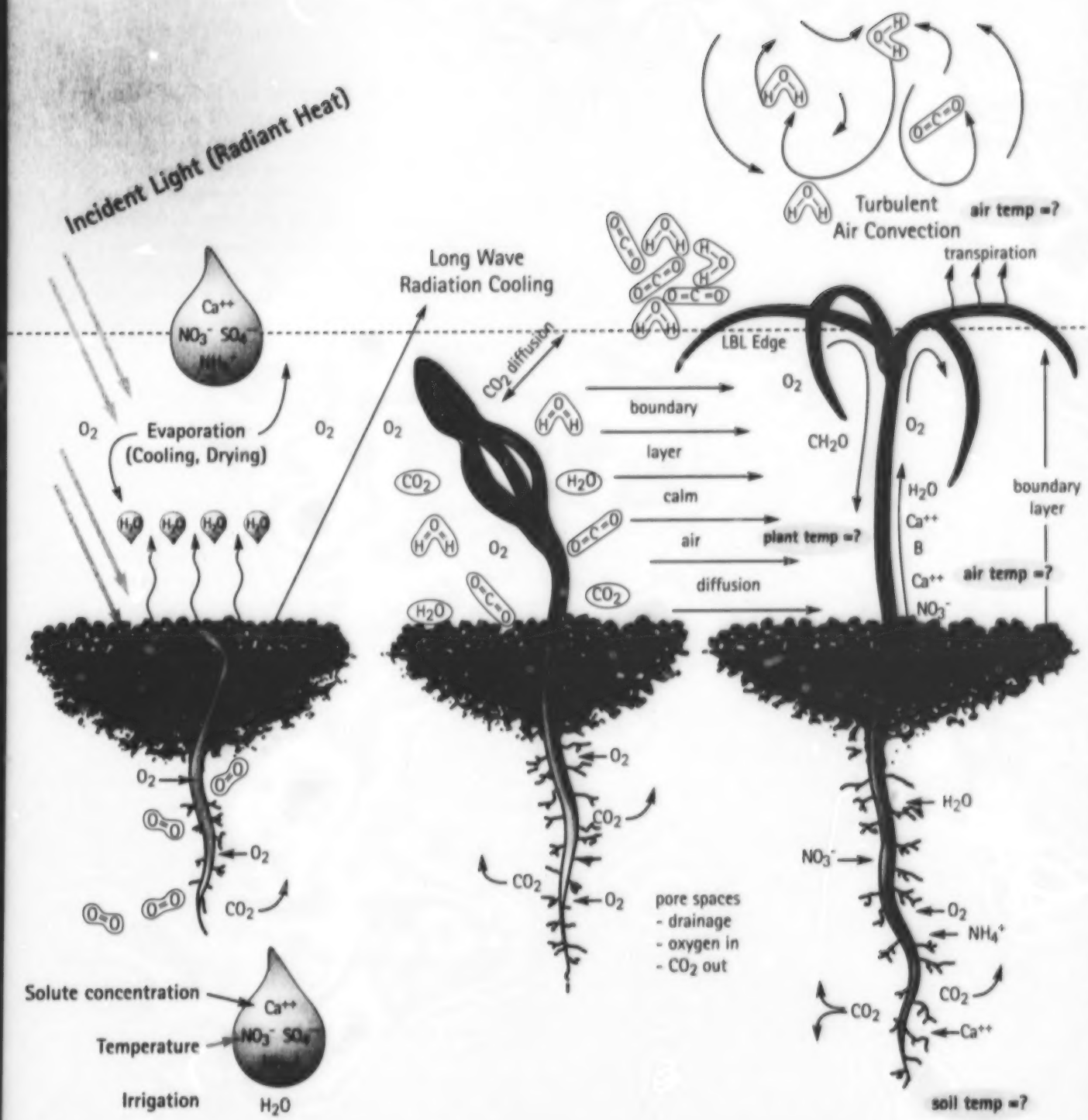
Incident Light (Radiant Heat)
Turbulent
Air Convection
air temp =?
transpiration
Long Wave
Radiation Cooling
LBL Edge
CO_2 diffusion
boundary
layer
calm
air
diffusion
plant temp =?
O_2
CH_2O
H_2O
Ca^{++}
B
NO_3^-
boundary
layer
air temp =?
Evaporation
(Cooling, Drying)
CO_2
pore spaces
- drainage
- oxygen in
- CO_2 out
NH_4^+
soil temp =?
Solute concentration
Temperature
Irrigation

Figure 96

Moisture

Water is essential for all life processes, and is one of the primary driving forces of germination. Successful stratification is dependent on specific moisture contents. After stratification, germination relies on unimpeded access to water. Water is key to imbibition, activation of the metabolic machinery, and radicle emergence.

Water potential is a measure of how "free" or easily extractable water is. It is measured in units of pressure (Mega-Pascals). Pure water has a defined water potential of zero (0.00 MPa), making it the easiest to extract or obtain. Adding solutes (dissolved substances such as fertilizer salts, for example) lowers the water potential below zero, effectively making water less "free" or more difficult to extract.

As water is preferentially extracted from the soil/growing media by transpiring plants and/or surface evaporation, the solute concentration rises, reducing water potential or the ease with which it can be extracted. For this reason it is important to monitor water quality during the growing cycle so that it can be replenished or replaced prior to becoming unavailable.

Water potentials also govern seed moisture uptake, and although dry seeds can muster very low water potentials (-100 MPa) with which to draw moisture and start imbibition, equilibrium can occur readily if water becomes more difficult to acquire. This can slow water uptake to the point of preventing complete imbibition and disrupting germination. So while we may want water with moderate solute levels with which to prime seeds, once seeds are at the "germination starting gate" and we want germination to proceed, water should be more "free" (closer to 0.00 MPa water potential).

...seeds are like sponges, competing for H_2O with solutes in the water

In summary, seeds are like sponges, competing for H_2O with solutes in the water, which behave like many little sponges. If there are too many solute particles present, seeds are unable to compete and obtain enough water with which to commence or continue the germination process (Figure 97).

Some ways in which water stress can be imposed on the embryo/germinant are through incomplete initial imbibition, dry media, high solute levels in the applied water, high evaporative demand causing drying, and pelleting. The coating used for pelletizing seeds competes very effectively for moisture with the seed coat itself when water becomes limiting. These conditions cause slow, non-uniform germination and reduce total GC.

Oxygen

Oxygen availability is extremely important because it is necessary for respiration, which provides the energy required for maintaining metabolic processes of seeds during stratification and germination.

Respiration = $C_6H_{12}O_6 + 6O_2 >>> 6CO_2 + 6H_2O$ + energy

Good gas exchange between the germinating medium and the embryo is essential. If oxygen concentration falls substantially below 21% (concentration in ambient air), germination of most seeds is inhibited (Khademi et al. 1992). Thus O_2 supply to and CO_2 dissipation away from the embryo are essential. Both are limited by physical properties of growing media, grit, water management practices, type of seed coat or pellet if pelletized, and sowing depth. Excess moisture hinders gas exchange between a seed and the external environment with possible negative impacts on respiration rate. Oxygen diffusion rate is ~10,000 times faster in air than water. This is why seeds can literally drown! Low oxygen environments are also conducive to root diseases.

Oxygen diffusion rate is ~10,000 times faster in air than water

Some common causes of poor oxygen/CO_2 diffusion rates are over-watering (frequency and/or duration), very fine or compacted growing media, excessive sowing depth, fine packing grit cover, and algae forming impermeable layers on growing media/grit surfaces.

Vigour is the ability to deal with unfavourable conditions and varies between species and seedlots. However, it also varies between seeds within a seedlot. During unfavourable conditions, such as low O_2 or temperature, it may be expressed as reduced crop uniformity (e.g., seedlot portion grown at 15°C in Figure 94).

Figure 97 Seed vs. solute - the battle for water.

Under optimum conditions most seeds germinate quickly. A low O_2 environment impacts those seeds having a higher energy requirement most—they will be slower to germinate (germination rate) or they may run out of energy and die (affecting germination %).

Oxygen availability can thus affect:

- germination rate
- germination capacity
- seed/embryo viability
- germinant viability.

Temperature

Assuming dormancy is overcome and moisture, oxygen, and carbohydrate reserves are not limiting, the rate at which biochemical processes proceed within a seed depends on seed temperature. The function that describes how the rate of a biochemical reaction changes with changing temperature is called the "Q_{10} factor" (Keeton 1972; Lehninger 1977). Over a specified range, it describes how the rate of a chemical reaction changes per 10°C change in temperature.

Between 5°C and 35°C for biochmical reactions in plants, the Q_{10} factor is approximately 2. This is an exponential relationship. This means that over the specified temperature range, a 10°C increase produces a doubling of the respiration rate (Figure 98).

Respiration of stored seed reserves fuels germination. Respiration rate thus approximates germination speed, governed by temperature. Practically speaking, each 1°C rise in seed temperature effects a 10% increase in respiration rate or germination speed. Going from 5 to 15°C, 10°C to 20, or 15 to 25°C doubles respiration rate, hence going from 5 to 25°C quadruples it! When balancing heating cost against the benefits of germinating at higher temperatures this exponential function becomes important. Because respiration/germination rates are higher at elevated temperatures, a 10% increase in respiration/germination rate effected by a 1°C temperature rise is much larger and potentially more beneficial when starting from a higher instead of lower initial temperature.

...each 1°C rise in seed temperature effects a 10% increase in respiration rate or germination speed

Raising germination temperatures into the 20 to 25°C range can provide substantial gains in terms of reduced crop cycle growing time and gains in crop uniformity and disease escape. However, does it pay, especially at high per unit energy costs?

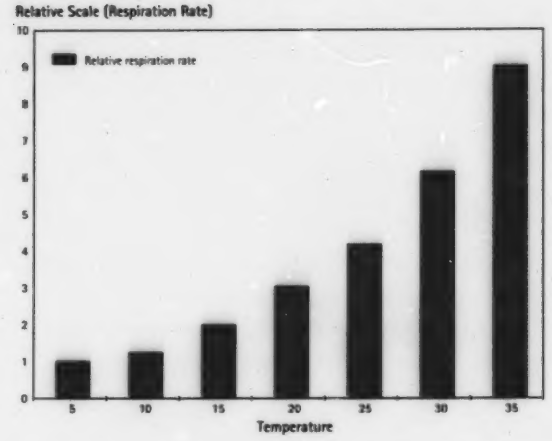

Figure 98 Plant respiration increases exponentially with temperature, having a Q_{10} of 2 between 5 and 35°C.

The cost of raising growing facility temperature is a function of the area of the structure, heat loss value of the covering, air exchanges per unit time, and outside temperature/wind/precipitation conditions. Generally speaking, greenhouse heating is a linear function between temperature and heating cost (Figure 99). This suggests that the heating cost of a 1°C rise in greenhouse temperature is approximately the same regardless of the starting point.

Combining Figures 98 and 99 gives the following (Figure 100).

Figure 100 demonstrates that with each successive increase in greenhouse temperature, the return on the heating investment increases (in terms of increased germination speed). In the above scenario (6 mm single poly at -10°C outside temperature), the first unit of heating energy is consumed to achieve a greenhouse temperature of 5°C. Relative respiration (germination) rate is at 1. Adding a second unit of heating

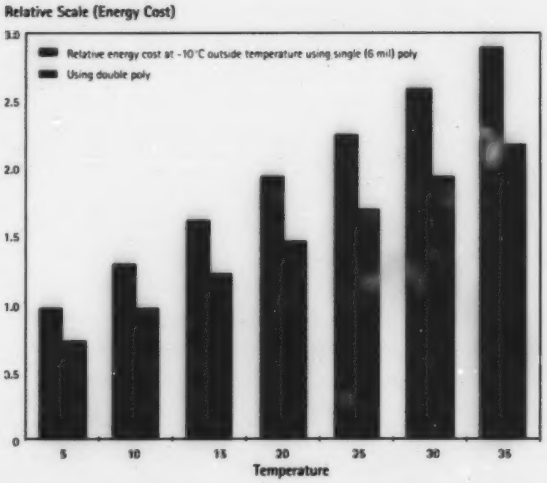

Figure 99 The relative greenhouse heating costs using single and double polyethylene roofing material.

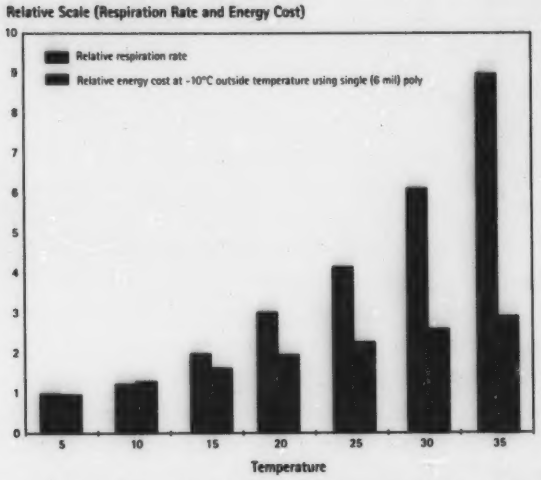

Figure 100 A comparson of the gains in respiration rate (approximating germination speed) and energy costs with changes in germination temperature.

energy brings greenhouse temperature to 20°C and effects a respiration rate of 3. Adding a third unit of heating energy brings greenhouse temperature to 35°C and raises respiration/germination rate to 9!

To summarize the above, it pays to increase temperature because of gains made in uniformity, germination speed and disease escape. In addition, because increased germination speed allows for a reduction in crop cycle time and total heating time, the higher the per unit energy cost the more it pays to increase germination temperature.

To increase fuel efficiency in poorly insulated buildings such as greenhouses, it makes sense to modify temperature management strategies depending on the prevailing weather. Since total heat sum appears to govern germination speed (Edwards and Leadem 2000), it is possible to make up for cool periods with warm ones. For example, during very windy weather consider using a lower heating setpoint, then make up for it during calm periods with higher temperatures (less heat loss due to convection). Those able to employ black-out screens as **energy curtains** may find the night a cheaper time to heat, allowing maintenance of a higher average germination temperature at a lower cost (less long wave radiative heat loss, smaller volume of air to heat, gain an insulating air layer). Remember that greenhouse air temperature does not necessarily equate to seed temperature. On sunny days grit temperatures can be substantially higher than air temperature, possibly warranting irrigation to cool the germinating seed. On clear nights, in a polyethylene house without an energy curtain, long wave radiative heat loss can easily drop growing media temperature 3 to 5°C below air temperature. Sensors should be installed in the growing media close to the seeds to estimate seed temperature. Monitoring seeds along with greenhouse air temperature on a continuous basis allows determination of the optimum heating strategy for a particular germination facility. It will also instill an appreciation of the impact of irrigation/misting cycles on seed temperature, mainly cooling, which may warrant more careful attention to humidity and water management strategies.

...total heat sum governs germination speed

A positive day/night differential of up to 10°C is recommended by some but found unnecessary by others. The theory is that it is an evolutionary trait developed to prevent deeply buried seeds from germinating. Only surface soil temperatures experience a significant day/night differential during spring, signalling the ability to make it to the surface should germination be attempted.

It can be said that root and shoot growth is an extension of germination, hence temperatures that promote good growth generally promote good germination. However, for many plants optimum germination temperatures are somewhat higher than optimum growing temperatures. This may be due to the fact that energy requirements for germination are generated from respiring storage reserves and germination-type growth involves primarily a reactivation and "unfolding" of previously developed systems and structures. Photosynthesizing organs have maintenance energy requirements that increase exponentially with temperature. The latter leads to the concept of "net growth" which equals photosynthetic production minus respiratory maintenance requirements (Figure 93).

..."net growth" equals photosynthetic production minus respiratory maintenance requirements

To facilitate rapid germination, low to mid 20°C seed temperatures are recommended. This allows the germinant to be transferred from a *germinating* environment to a *growing* environment sooner. A germinating environment employs continuous warm, low light, low to zero nutrient, low **vapour pressure deficit** (high **relative humidity**), and high soil moisture conditions. Maintaining these conditions after germination increases the risk of contracting fungal diseases, and leads to extremely soft plants, incapable of resisting stress in general.

Light

Some conifer seeds require light to germinate, but the seeds must be fully imbibed and the required intensity is low, 1–5 lux (equivalent to bright moonlight) (Leadem 1996). The red light (660 nm) portion of the spectrum stimulates germination and the far-red portion (730 nm) inhibits germination. Growers in BC have used this strategy with some success

when incompletely stratified seeds have had to be sown in an emergency. Basically it involves applying the seed cover (grit) several days to a week after sowing the seeds, thereby allowing light to help initiate the germination process. The red/far-red ratio impacts on various plant growth characteristics, hence may be worth investigating with respect to germination rate and capacity.

The recommended photoperiod is a 20-hour extended day to help reduce **etiolation** (stretching) of the **hypocotyl** after germination and prevent premature budset in northern seedlots. Etiolation increases with a reduction in the red/far-red ratio. During the winter months, when light intensity, quality, and natural photoperiod are lowest, etiolation can be excessive, resulting in very weak, spindly plants.

Sunlight warms surfaces it can reach, altering the boundary layer climates associated with them. This is beneficial in most cases but will induce horizontal temperature gradients between shaded and non-shaded sections in the propagation area. Exposed plant parts will warm and growth rates increase relative to shaded parts or other plants, something to keep in mind when considering uniformity. Artificial light generates heat as well but the degree to which it warms surfaces depends on its intensity, spectral distribution, and distance from the object in question. When targeting 8 to 10 foot-candles (~80 to 100 lux) for photoperiod extension this contribution is generally not significant. However, seedlings growing directly under individual lights may display enhanced growth, especially if lights are installed close to the crop.

Seedlots with low vigour have been known to benefit from reduced grit depth. Part of this response may be due to increased light and oxygen penetration through to the seeds, or increased seed temperature if the intensity of light is high enough. Mostly it is attributed to reduced physical resistance to "emergence" above the seeds.

Relative Humidity

Assuming complete imbibition (*and moist/wet growing media*), the relative humidity near a seed (laminar boundary layer) during stage 1 needs to be as close to 100% as possible. Free water on seeds should be limited to a film, which will act as insurance against loss of moisture from within the seeds while not limiting gas exchange. Under no circumstances should seeds be submersed or "floating" for extended periods of time. During germination stage 1 seeds only take water up for various metabolic and physical processes. No transpiration is taking place, required, or even possible, hence any substantial vapour pressure deficit near seeds will only cause them to dry.

During stage 2 the radicle has emerged and cotyledons are turning green and unfolding, signalling the presence of chlorophyll and the ability to activate the photosynthetic process upon exposure to light. Transpiration of water vapour quickly increases in importance as the seedling needs to begin taking up water and nutrients from the soil solution, evaporatively cool itself, and maintain stomatal function. As soon as this stage is evident in the majority of the crop it is imperative that relative humidity levels are dropped (slightly) to allow transpiration to commence. Maintaining a stage 1 environment to allow late germinants to catch up can compromise early germinants. A judgement call eventually has to be made. Below ground the radicle needs to encounter an environment conducive to growth as well. Access to oxygen, mineral nutrients and water is imperative. Maintenance of gas exchange and temperature requires careful attention to irrigation management.

Evaporating water is one of the most effective cooling mechanisms... and can reduce the speed of germination

Relative humidity can influence the temperature of a moist surface and its LBL through the process of evaporative cooling. Evaporation occurs when there is a vapour pressure gradient between a moist surface and its surroundings—in this case the seeds, grit or media surface, and the surrounding air mass. Evaporating water is one of the most effective cooling mechanisms (e.g., goose bumps after climbing out of the lake on a hot summer day!). If relative humidity is low during stage 1, evaporation will occur from the seeds, grit, and media surface, thereby cooling them and the laminar boundary layer. This will reduce the speed of germination (respiration/growth rate) and may lead to reduced uniformity. Boundary layers and surfaces have been found to be as much as 5°C cooler than the adjacent air mass.

Besides cooling, any drying can effect a water stress, slowing or stopping the germination process. This would need to be overcome with extra misting or watering, resulting in additional cooling, and possibly a reduction in gas exchange for a period of time. Extreme desiccation will damage the emerging radicle or cause death of the germinant.

As the young seedling continues to grow, only a portion of it remains in the horizontal LBL above the container or growing media surface. If the boundary layer is much cooler than the media, adjacent air, or both, condensation can form on lower plant parts, encouraging disease organisms. Cooler plant parts function slower than warm plant parts, hence a bottleneck effect can occur in the stem (or roots) with respect to plant fluid flow. This can seriously compromise current and future seedling growth rate and function (e.g., early blossom-end rot of tomatoes).

There are differences between lab and nursery germination worth discussing. Lab germination is carried out in a very controlled and sterile environment. A seed cover is not applied and temperatures are generally slightly higher and more rigidly controlled than those employed at the nursery. In the lab a seed is defined as germinated when the radicle has extended to four times the seed length. In the nursery a germinant is generally counted once it has emerged from the seed coat and its cotyledons are unfolding and photosynthesizing. By this time the radicle is approximately 10 times the seed length. Basically, at the nursery the judgement call for germination is made later in seedling/germinant development (Figure 101).

...at the nursery the judgement call for germination is made later in seedling/ germinant development

One scenario, which is more common for some of the *Abies* species, is that a seed may be capable of producing a radicle but may not have the energy to shed its seed coat or it may grow abnormally once it does, perhaps not expressing positive **phototropism**. This can lead to significant reductions in yield at the nursery relative to predictions made based on lab germination.

Seedlots are tested for both germination capacity (GC) and germination speed at the BC Ministry of Forests Tree Seed Centre and retested at specific intervals (see Table 7, page 51). Every effort is made to provide accurate, up-to-date information, but it is not possible (or necessary) to test all stored seedlots each year. Results of the most recent test can be viewed on SPAR. They are also provided on request labels which accompany sowing requests.

Germination tests are based on a random sampling of seeds from a seedlot. Seeds for a particular sowing request are not randomly sampled since all seeds for a request may come from one box even though the seedlot may be 20 boxes in size. It is important to note then that variable germination can occur due to differences in sampling for testing and sowing request withdrawal procedures. All new seedlots are blended (mixed to reduce variability) prior to long-term storage, but this practice never eliminates all of the variability within a seedlot.

The Tree Seed Centre has been conducting a Quality Assurance (QA) program on sowing requests since 1992. This entails taking a seed sample from sowing requests just prior to shipping.[8] Each year approximately 200 sowing requests are sampled for moisture content (Table 8, page 56) and GC.

Nurseries are subsequently asked to provide the GC they attain operationally. Nursery results are then compared to laboratory and QA test results, and presented as falldowns relative to current laboratory germination (Table 14). In Table 14, falldowns are indicated as negative values, compared to lab germination, and increases in GC are presented as positive values. The positive values most likely arise from sampling or treatment differences.

The overall falldown of all sowing requests tested prior to shipping, sampled between 1992 and 2001, at the Tree Seed Centre is 2% below lab germination. The majority of this

[8] Quality assurance grams are added to selected requests prior to imbibition or pelletting.

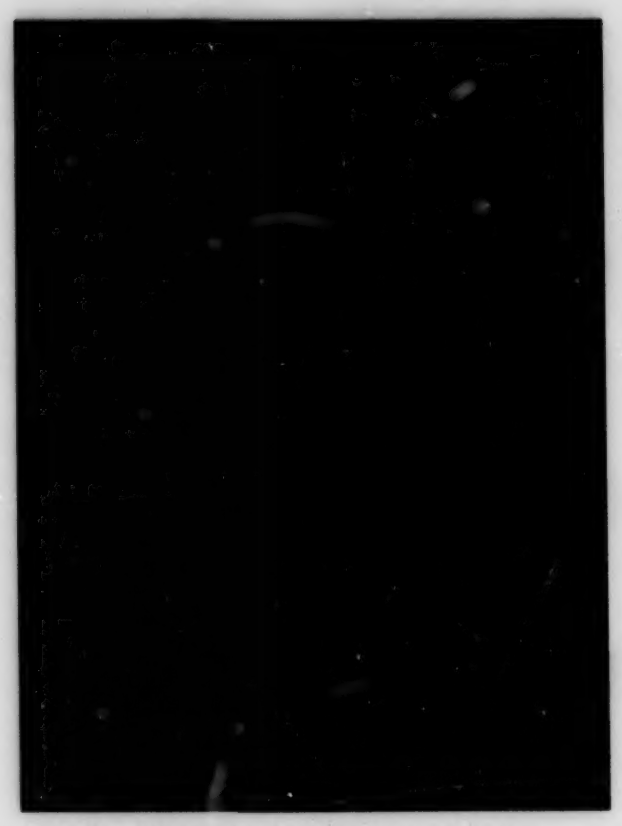

Figure 101 Different criteria for quantifying germination exist between the nursery and the lab.

Table 14 Germination capacity (GC) falldowns relative to latest standard lab germination test, at time of shipping and at the nursery. Sample sizes (#), mean GC and estimate of falldown presented for each.

Species	Tree Seed Centre at shipping			Nursery information		
	#	Mean GC (%)	Falldown (%)	#	Mean GC (%)	Falldown (%)
Amabilis fir	138	66	2	48	72	-2
Grand fir	48	76	1	22	68	-7
Subalpine fir	106	62	-10	51	68	-6
Western redcedar	364	73	-7	126	75	-3
Coastal Douglas-fir	145	92	0	51	89	-3
Interior Douglas-fir	165	89	1	58	88	-2
Mountain hemlock	41	91	2	10	84	-3
Western hemlock	133	89	-2	56	82	-8
Western larch	183	76	-4	40	89	6
Coastal lodgepole pine	50	92	1	6	91	3
Interior lodgepole pine	323	93	0	102	86	-6
Western white pine	167	59	-22	73	73	-10
Ponderosa pine	113	86	-2	13	85	-7
Sitka spruce	73	93	-1	9	87	-7
Interior spruce	411	88	2	118	88	2
Sitka × interior spruce hybrid	42	90	2	0	0	0
	2502	82	-2	783	77	-3

difference can be attributed to subalpine fir and western white pine, which are high priority species for improvement in seed preparation techniques at the Tree Seed Centre. Western redcedar also displays large falldowns, but a component of this can be attributed to the pelletting process, which is estimated to delay germination by up to four days.

The falldown at the nursery is, on average, surprisingly low at 3% below lab germination. Western white pine showed the largest nursery falldown and is a high priority for improvements in operational stratification techniques. Western hemlock exhibited an 8% decline, followed by grand fir, ponderosa pine and Sitka spruce, which exhibited 7% declines in the nursery compared to SPAR GC. A falldown of 6% for interior lodgepole pine is surprising and considered high for a species which generally exhibits very rapid and high GC. A possible explanation is that, due to the excellent germination characteristics of lodgepole pine, it is sometimes sown outdoors under generally sub-optimal conditions. In general there are few complaints about poor germination of lodgepole pine crops, but it is very important to be as efficient as possible with this species due to significant shortages of available select (orchard produced) seeds.

These falldown figures are intended as guidelines and provincial averages. They help establish priorities for the cone and seed improvement program for the Tree Seed Centre and its clients. Best estimates for a particular nursery are based on the values calculated as part of its QA program. All nurseries are encouraged to perform at least some germination counts following sowing to determine their actual falldowns, if any. Seed owners are becoming more frugal with seeds and a proper QA program will help determine what can and cannot be done to meet their expectations.

The falldown at the nursery is, on average, 3% below lab germination

Seeds sown at the nursery may be subjected to a longer stratification period as sowing requests are shipped after the appropriate stratification duration, but seeds are generally placed in a cooler at the nursery until sowing, thereby extending the stratification period. This may improve germination characteristics if a seedlot is more dormant than average for the species. However, it may decrease germination if the seedlot is of poor quality and extended stratification causes deterioration or

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fungal build-up. Germination in the lab is based on a 21 or 28 day test, but nursery counts often extend much longer. Part of this is due to the difference in classification of a germinant, but it may also include late germinants that were not included in the lab test results. Nurseries also may perform additional treatments on their seeds such as density separation, soaking, priming, or sanitation soaks (H_2O_2). These practices can change the quality of a sowing request (positively or negatively), confounding the comparison with lab test results.

Conditions in a nursery are generally more variable and can be more extreme than in a germination cabinet. Because the nursery environment is more variable and less sterile, weaknesses that might go unnoticed in the lab can be expressed and lead to losses in the nursery. The fact that high vigour seeds generally achieve higher GC under a wider range of temperatures than low vigour seeds is a classic example. Temperature is an obvious factor and will generally be more stable under lab conditions. However, one has to take into account the humidity and light intensity conditions in order to assess the full impact of various temperature regimes.

Once completely stratified and fully imbibed, temperature (heat) sum becomes one of the most important factors governing germination speed. The distribution of this temperature (day/night) is not critical to the germination process (Edwards and Leadem 2000). However, to accurately compare germination strategies, temperature regimes should be converted to heat sums.

Germination Counting in the Nursery

There is no standard method for performing nursery germination counts. Nurseries vary on the use of half- or full-block counts. Also, the criteria for sampling, defining germination, count duration, and number of blocks/half-blocks used to provide a reasonable estimate of GC varies by nursery.

It is recommended that full blocks are used since seeding machinery, if it displays a problem that results in empties, will do so with a pattern that repeats itself on a block-by-block basis. For some equipment, counting half-blocks can result in missing a seeder problem (it depends on how many drops or rolls a seeder makes per block). Another reason for sampling whole blocks is that the trend to larger cavities reduces the number of seedlings to be counted. Also, some of the larger containers, such as the 77 cavity container, cannot be equally split to allow counting half the cavities.

It is difficult to come up with firm guidelines, as a database that looks at this level of sowing detail does not exist. However, very general recommendations are that germination counts in the nursery should be based on at the very least two blocks and a maximum of 2% of the blocks, depending on request size, species and crop uniformity.

Extra Seed

Nurseries can have seeds left over after sowing and may wonder what to do with them. All extra seeds should be returned to the BC Ministry of Forests Tree Seed Centre. Storage of seeds destined for Crown land reforestation must be stored at the Ministry's Tree Seed Centre. To increase the probability of salvaging seeds, as a customer service nurseries should dry them down to a lower moisture content and return them to the Tree Seed Centre. Seeds should be redried and sent in a timely manner, in an appropriate container, with seedlot identification (sowing request labels) included.

At the Tree Seed Centre, returned seeds are promptly evaluated for condition (obvious deterioration, mould, physical damage, pre-germination, or high moisture content), amount returned, class of seed, time since stratification was completed, and seedlot balance. Seeds that are obviously deteriorated are discarded. It should be emphasized that the main cause of deterioration in returned seeds is the maintenance of a very high (30%+) moisture content for prolonged periods. In general, the moister the seeds, the more rapidly they deteriorate.

If seeds are considered salvageable they are sampled for GC and moisture content and dried back to a storage moisture content (4.9–9.9%). If GC is poor the seeds are discarded. If GC is relatively unchanged, the seeds are placed into freezer storage for an additional six months and then retested for GC. If GC is still comparable to the parent seedlot, the seeds may be blended into the parent seedlot or stored as a separate seedlot for use in future sowing requests.

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Additional Information

Suggested Reading

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R.E. Farmer, Jr. 1997. St. Lucie Press.
Delray Beach, FL. 253 pp.

Seed and Seedling Extension Topics.
A newsletter published twice a year. For information on receiving this Newsletter or contributing articles contact Diane Gertzen (604) 930-3303.
Diane.Gertzen@gems3.gov.bc.ca

Tree and Shrub Seed Handbook.
A.G. Gordon, P. Gosling and B.S.P. Wang [Eds.].
The International Seed Testing Association, Zurich, Switzerland. 1991.

Tree Seed Working Group Newsbulletin.
Published twice a year. For information on receiving the Newsbulletin or submitting articles, please contact Dave Kolotelo.

Web Links of Interest

AOSA – Association of Official Seed Analysts, Inc.
http://www.aosaseed.com

Danida Forest Seed Centre
http://www.dfsc.dk

Forest Nursery Notes
http://www.na.fs.fed.us/spfo/rngr/fnn_list.htm

ISTA – International Seed Testing Association
http://www.seedtest.org

IUFRO Unit 7.03.04
Diseases and insects in forest nurseries
http://iufro.boku.ac.at/iufro/iufronet/d7/hp70304.htm

IUFRO Unit 2.09.00
Seed physiology and technology
http://iufro.boku.ac.at/iufro/iufronet/d2/hp20900.htm

Nursery Technology Co-operative
Oregon State University
http://www.cof.orst.edu/coops/ntc/ntc.htm

Pest Management Regulatory Agency
Health Canada
http://www.hc-sc.gc.ca/pmra-arla/english/index-e.html

Reforestation, Nurseries and Gene Resources
US Forest Service
http://www.na.fs.fed.us/spfo/rngr/index.htm

Seeds of Woody Plants in the United States
The long awaited revision to the classic text is continuing, but finished chapters to date can be viewed at http://wpsm.net

Southern Forest Nursery Management Cooperative
http://www.forestry.auburn.edu/sfnmc/sfnmc.html

For information on other cone and seed insects, visit:
www.for.gov.bc.ca/TIP/IIG
www.for.gov.bc.ca/TIP/IID

Contacts

If you are unfamiliar with SPAR and require access or training, contact:
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Company name	Address, phone/fax; e-mail; contact person	Product/service
Aqua-Pak Styro Containers Ltd	7398 132 St Surrey BC V3W 4M7 590-2886/590-8412 (fax)	Styro cartons; boxes
Argus Control Systems Ltd.	1281 Johnston Rd. White Rock BC V4B 3Y9 (604) 538-3531 argus@argusd-controls.com www.argus-controls.com	Kiln control system
Arrolo Product Handlers	1040B Coulter Ave Winnipeg MB R3E 0X8 (204) 783-5000	Cleaners; Clipper; Secondary cleaners (Micro III)
BCC Silviculture Systems	Profilgatan 15 S-261 35 Landskrona Sweden 011-46-418-449920, 011-46-418-29185/-449922 (604) 669-8333/(604) 669-7173 (Marketing admin.)-411144 9 Techn. dept., serv.)(fax) Vancouver Contact: Justin Elvin Jensen (604) 669-8333/(604) 669-7173	Cone & seed handling; Seedling production; Planting; Hilleshog Note: e-mail: bcc@bccab.com Web: http://www.bccab.com
BC Scale Company Ltd.	5920 – 200A St. Langley BC V5A 5E6 (604) 534-8633	Balances, sales and service
BDH Inc.	350 Evans Ave. Toronto ON M8Z 1K5 (416) 255-8521	Talc
Beaver Plastics Ltd	12150 160 ST Edmonton AB T5V 1H5 (403) 453-5961/(403) 453-3955 (fax)	Box liners, Plastics; Styroblock® containers
BM & M	9377 – 193 St. RR 34 Surrey BC V4N 4E7 (604) 888-8400 www.bmandm.com	Screening machines
Buyers Packaging Group Ltd	7533 Progress Way Unit 5 Delta BC V4G 1E7 (604) 940-6513/6519 (fax)	Foam wrap for packaging: Celle air; containers; Tharco corrugated boxes
Bevco	9354 194 ST Surrey BC V3T 4W2 (604) 888-1455	Conveyors
C & E Mesh Products	929 Tupper Ave Coquitlam BC V3K 1A4 (604) 524-3606/(604) 525-2133 (fax)	Screens
Coe Manufacturing Canada Inc	15100 River Rd Richmond BC V6V 1L5 (604) 276-1722/(604) 276-2160 (fax)	Kiln supplies & service; Moore dry; Wet bulb wicks (12/pkg)
Controlled Environments Ltd	590 Berry St Winnipeg MB R3H 0R9 (204) 786-6451	Conviron Germinator
Crippen Manufacturing Company Inc	Alma MI 48801 USA (517) 463-2119	Grain & seed cleaning & conditioning equipment; Clipper; Secondary cleaners, parts orders, supplies
Crown Paper	PO Box 70 Saanichton BC V0S 1M0 (250) 526-0848	Bags, poly
Fandrich Cone Harvesters Ltd.	2461 Sunnyside Place Abbotsford BC V2T 4C4 (604) 850-0666 e-mail: helmut@fch.wimsey.bc.ca	Aerial cone rakes, desailers, grapples
Fisher Scientific	196 West Third Ave Vancouver BC V5Y 1E9 (604) 872-7641; sales rep direct line: (604) 205-5703/5710 (fax) http://www.fishersci.ca	Lab supplies; Balances, Filter paper; Thermometers; silica gel; buffer solutions, timers, gloves

Company name	Address, phone/fax, e-mail, contact person	Product/service
General Fasteners	#3 - 1678 Fosters Way Annacis Island Delta BC (604) 526-1606/1608 (fax) (604) 523-2305	Poly bags; Strapping for the Signode strapping machine
Hance Corporation, The	235 East Broadway Westerville OH 43081 USA	Scalper
Happel, Carl	8293 Old Kamloops Rd Vernon BC V1H 1W8 (250) 558-0746/(250) 558-0764 (fax) Seed-Pelleting@telus.net	Seed pelleting
Hoffman Manufacturing Co	325 11TH Ave SE PO Box 547 Albany OR 97321 USA (541) 926-2920/(541)926-3949 (FAX) Toll-free: 1-800-692-5962 International fax:1-800-343-6724	Blotting paper; Germination dishes (4 5/16" x 4 5/16" x 1 1/8" Rectangular with Lids); Sample cups
Kimberly-Clark Corp	PO Box 2001 Neenah WI 54956 USA Toll free: 1-800-558-5066	Kimpak, 4" x 4", Type K-22, Ply 20
Mettler	8559 Main St Vancouver BC (604) 263-6593	Balances & scales
Pioneer Packaging	1584 Highway 41A North Dixon KY 42409 USA (502) 639-9133	Germ dishes (9/sc 4 5/16" x 4 5/16" x 1 1/8")
Prairie Sun Sales & Service Ltd	PO Box 1840 Rosetown SK S0L 2V0 (306) 882-4040	Cleaners (parts); Specific gravity separators; Clipper; Parts distribution
Prunella Technics OY	PO Box 6 Peltosuontie 2 41330 Vihtavuori Finland +358-9-41-771-077/+358-9-41-771-798 (fax)	Seed extraction equipment (PRUTEC system)
Purve's Ritchie Equipment Ltd.	5175 Regent St. Burnaby BC V5C 4H4 (604) 299-5888	Dewingers, cement mixers, fans
Quality Seed Collections Ltd	PO Box 144 Blind Bay BC V0E 1H0 (250) 675-2463/(250) 675-2202 (fax)	Cone cutters;
Salton Fabrication Ltd	19087 96 Ave Surrey BC V4N 3P2 (604) 888-0122/888-2959 (fax)	Kiln manufacturing service; charts; kiln firebox; Hot water & steam boilers; Heat transfer systems; Wood-fired boilers
Seedtech Systems	5050 Laguna Blvd., Suite 112-333 Elk Grove, CA 95758 USA (916) 684-1196 Fax (916) 684-7675	Seed processing equipment
Seedburo Equipment	1022 West Jackson Blvd Chicago IL 60607-2990 USA 1-800-284-5779/(312) 738-5329 (fax)	Bag holders, Mosher, Magnifier lamp; Pneumatic separators
Spiroll Kipp Kelly	1320 Church Ave Winnipeg MB R2X 1G4 (204) 694-3084	Specific gravity separators
Steinmetz, F G R Inc	13668 Hilton Rd Surrey BC V3R 5J7 (604) 584-8224/2795 (fax)	Microscopes, new/used/repairs; Fibre optic light source
Unitrend Plastics Manufacturing Ltd	7351 Progress Place Delta BC V4G 1A1 (604) 940-8900/940-8901 (fax)	Poly - Plastic bags (4mil, 12" x 20", 17" x 28", 27" x 40")
Western Canadian Screens	669 Derwent Way New Westminster BC V3M 5P7 (604) 520-3073/522-5949 (fax)	Hardware cloth (4" x 4" mesh, 23 gauge); Tarps; Canvas; Industrial cloth/fabrics/plastic/poly
Yellow Point Propagation Ltd	13735 Quennell Rd RR 3 Ladysmith BC V0R 3E0 (250) 245-4635 (250) 245-5935	Air separators
Zero Pak Products Ltd	2471 Simpson Rd Unit 160 Richmond BC V6X 2R2 (604) 278-4828/278-2636 (fax)	Ice packs seed transport; Industrial gel ice

& Abbreviations of BC Conifer Species

Amabilis fir	Ba	*Abies amabilis* (Dougl.) Forbes
Grand fir	Bg	*Abies grandis* (Dougl.) Lindl.
Subalpine fir	Bl	*Abies lasciocarpa* (Hook) Nutt.
Noble fir	Bn	*Abies procera* Rehd.
Coastal Douglas-fir	Fdc	*Pseudotsuga menziesii* (Mirb.) Franco
Interior Douglas-fir	Fdi	*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco
Mountain hemlock	Hm	*Tsuga mertensiana* (Bong.) Carr.
Western hemlock	Hw	*Tsuga heterophylla* (Raf.) Sarg.
Western larch	Lw	*Larix occidentalis* Nutt.
Whitebark pine	Pa	*Pinus albicaulis* Engelm.
Limber pine	Pf	*Pinus flexilis* James
Coastal lodgepole pine	Plc	*Pinus contorta* Dougl.
Interior lodgepole pine	Pli	*Pinus contorta* var. *latifolia* Dougl. ex Loud.
Western white pine	Pw	*Pinus monticola* Dougl. ex D. Don
Ponderosa pine	Py	*Pinus ponderosa* Laws
Black spruce	Sb	*Picea mariana* (Mill.) B.S.P.
Sitka spruce	SS	*Picea sitchensis* (Bong.) Carr.
Interior spruce	Sx	*Picea glauca* (Moench) Voss, *Picea engelmannii* Parry ex Engelm and hybrids
Sitka × interior spruce hybrid	SxS	*Picea x lutzii* Little
Yellow-cedar	Yc	*Chamaecyparis nootkatensis* (D. Don) Spach
Western redcedar	Cw	*Thuja plicata* Donn ex D. Don

Aflatoxins: Virulent toxin produced by the Hyphomycetes *Aspergillus flavus* and *A. parasiticus*, growing on foodstuffs, especially nuts; highly carcinogenic.

Anatomy: The study of the structure of living organisms, especially of their internal parts by means of dissection and microscopic examination (compare *Morphology*).

Angiosperms: The flowering plants, which are the plants with the most advanced structural organization in the plant kingdom. Monocots with one cotyledon, dicots with two cotyledons (compare *Gymnosperm*).

Ascocarps: A fruiting body of fungi containing asci (singular ascus), a sac-like cell generally containing a definite number of ascospores.

Ascospores: Spores resulting from sexual reproduction borne in an ascus.

Aspiration: A drawing of something in, out, up or through, by or as if by suction.

Asymptomatic: Not showing any clinical manifestations.

Bareroot: The system for growing forest tree seedlings in harrowed fields. Seeds sown directly into soil or plants transplanted to soil for a portion of the crop cycle.

Casehardening: The incomplete opening of cones due to the kilning of very moist cones or caused by fungal or insect activity.

Chlamydospores: Thick-walled asexual resting spores typically formed by many soil-borne fungi.

Conidia: An asexual fungal spore usually formed at the tip or side of a sporogenous cell.

Conidiophore: A fungal hypha bearing conidiogenous cells from which conidia are produced.

Conophyte: Any insect that feeds or develops on or within conifer cones.

Container: Used to describe a system for growing forest tree seedlings. Seeds are sown in media-filled containers that will restrict root growth to the size and shape of the cavity in which seeds are sown.

Convection: The transfer of heat from place to place by the circulation of heated particles of a gas or liquid.

Corrosion cavity: The cavity in the central portion of the mega-gametophyte that forms through cell breakdown. The embryo will grow into this cavity.

Cotyledons (syn. seed leaves): The photosynthetic structures of the embryo* found in the seed and emerging during epigeal* germination. They also protect the shoot apical meristem.

Cupressaceae: A family of gymnosperms* characterized by persistent scale-like leaves and cones in which the bracts and scale is wholly fused. Genera present in BC include *Thuja*, *Chaemacyparis*, and *Juniperus*.

Damping-off: The killing of the seedling by micro-organisms before emergence from the soil (pre-emergence) or the collapse of the hypocotyl and/or radical immediately after emergence, usually at the groundline.

Deterioration: A general term used to describe the reduction in seed quality. Different physiological mechanisms may be involved.

Disinfected: The removal or killing of fungal propagules or hyphae once the organism has penetrated the seed coat.

Disinfest: The removal or killing of fungal propagules adhering to the surface of materials.

Dormancy (seed): The condition when mature, viable, imbibed and healthy seeds fail to germinate.

Dusts: A pesticide active ingredient mixed with finely-ground particles of inert materials such as talc, clay or volcanic ashes. They are used dry and the concentration of active ingredient is usually low (i.e., 1 to 10%).

Efficiency: A measure of the success (gain*) of a treatment or processing step in relation to the amount of waste material. An efficiency of 95% indicates only 5% of the seed was discarded in producing the achieved gain.

Embryo: The structure in plants that develops from the zygote prior to germination.

Emulsifible concentrates: Contain a pesticide active ingredient, one or more petroleum-based solvents and an emulsifier which allows the formulation to be mixed with water. When the spray mixture is prepared, the pesticide is suspended as minute droplets in the mixture.

Energy curtain: Any cover placed over an object to trap/reflect long wave radiation emitting from it. Used in greenhouses to achieve the above as well as reduce the volume of air requiring heating on cold nights, thereby reducing energy consumption.

Enzyme: A complex protein produced in living cells that speeds the rate of (catalyzes) a chemical reaction.

Epigeal: Describing seed germination in which the cotyledons emerge from the seed and function as leaves. Typical of gymnosperms.

Etiolation: A condition found in plants being grown in the dark or with greatly reduced light intensity. Symptoms include increased stem elongation coupled with poor leaf development and lack of chlorophyll.

Flowables, dry: Consist of a pesticide active ingredient mixed with inert ingredients to form granule-size particles. The particles are added to water, producing a suspension just like a wettable powder. The spray mixture must be continuously agitated. Dry flowables are easier to pour than powders and are less of an inhalation hazard during mixing.

Frass: Particulate insect excrement, similar to dry granular cereal, often mixed with bits of plant material. When found within cones, frass is usually indicative of the feeding activities of conophytic caterpillars.

* Indicates those words found elsewhere in the glossary.

Fungi: (sing. fungus) an undifferentiated organism lacking chlorophyll and conductive tissues.

Gain: The improvement in a particular trait (e.g., upgrading a seedlot from 85 to 95% germination is a 10% gain).

Genetic worth (GW): A measure of the genetic quality of a seed or vegetative lot over wild stand material, measured for a specific trait.

Gymnosperm: Any plant whose ovules,* and the seed into which they develop, are borne unprotected, rather than enclosed in ovaries. (The term gymnosperm means naked seed.)

Heteroconophyte (Faculative conophyte): Any insect that has no dependence upon conifer cones but opportunistically feeds upon them when they are available.

Hydrolysis: Chemical decomposition that changes one compound into other compounds by taking up water.

Hyphal: (of hypha, pl. hyphae) Threads of mycelium produced asexually by fungi.

Hypocotyl: The region of the stem beneath the cotyledons and directly above the root of an embryo. It grows rapidly in seedlings showing epigeal germination and lifts the cotyledons above the soil surface.

Imbibed: Seeds that have become swollen and physiologically active following the movement of water into them. Full imbibition is characterized by all seed contents becoming filled with water. It is critical that water reaches the embryo.

Inoculum: The spores, mycelium, sclerotia, or other propagules of a pathogen that initially infect a host or crop.

Integument: The outer protective covering of a plant ovule.* It is perforated by a small pore, the micropyle. Usually two integuments are present in angiosperms* and one in gymnosperms.* After fertilization the integuments form the seed coat.

Laminar boundary layer: The layer of air in contact and immediately surrounding a surface.

Lipids: An organic compound that is insoluble in water, but soluble in organic solvents.

Macroconidiospores: Conidiospores as distinguished from microconidia. Large asexual spores produced by fungi.

Megagametophyte: Female gametophyte.

Metabolic: Relating to the chemical processes that occur within a living cell or organism.

Microconidia: (sing. microconidium) Small asexual spores called conidia, produced by fungi.

Morphology: The study of the form and structure of organisms, especially their external form (compare *Anatomy*).

Mycelium: (pl. mycelia) A mass of hyphae: the thallus of a fungus.

Mycotoxins: A fungal secondary metabolite which is poisonous to man or animals.

Nucellus: The tissue that makes up the greater part of the ovule* of seed plants. It contains the embryo* sac and nutritive tissue. It is enclosed by the integuments* except for a small gap, the micropyle.

Nursery-handling factor: The factor used to ensure that nursery equipment has sufficient seed to enable sowing of all cavities. A factor of 0.2 seeds per cavity sown is used consistently for all container types.

Obligate conophyte: Any insect that must spend part of its life feeding or developing on or within conifer cones.

Orthodox: A classification of seed based on storability. These seeds can be dried down to low moisture contents (<10%) and stored under sub-freezing temperatures for extended periods (decades) (compare *Recalcitrant*).

Osmotic potential: Also termed solute potential, it carries a negative sign and refers to the change in free energy or chemical potential of water imparted by solutes dissolved in it.

Osmoticum: A general term for large molecular weight molecules used to regulate water uptake in seeds.

Oversow factor: The factor used to provide a "correction" of the requested number of viable seedlings, to account for non-productive cavities.

Ovules: The part of the female reproductive organs of seed plants that consists of the nucellus,* embryo* sac, and integuments.* The ovules of gymnosperms* are situated on ovuliferous scales of the female cones while those of angiosperms* are enclosed in the ovary. After fertilization, the ovule becomes the seed.

Parenchyma: Roughly spherical relatively undifferentiated cells, frequently with air spaces between them. The cortex and pith* are composed of parenchyma cells.

Parasitic: (of a parasite) The action of a parasite, an organism that lives at the expense of another, usually invading it and causing disease.

Photosynthesize: The ability to convert light energy to chemical energy. In green plants it refers to the ability to utilize chlorophyll in the presence of light energy to produce carbohydrate from carbon dioxide and water.

Phototropism: The growth of plant organs in response to light.

Phytotoxic: Poisonous or injurious to plants.

Pinaceae: A family of gymnosperms* characterized by persistent or deciduous spirally arranged leaves, distinct bracts, and scales in a woody cone. Genera present in BC include *Abies, Larix, Picea, Pinus, Pseudotsuga,* and *Tsuga.*

Pith: The cylinder of parenchyma* tissue found in the centre of plant stems interior to the vascular system.

Pneumatic separator (syn. Aspirator): A piece of equipment that separates seeds through the use of air currents based on terminal velocity.

Polyethylene: A lightweight plastic resistant to chemicals and moisture and used mainly in packaging.

Polyethylene glycol: A common type of osmoticum* used in seed priming treatments.

Procambium: A plant tissue formed by the apical meristems of shoots and roots. It consists of cells elongated parallel to the long axis of the plant. The procambium subsequently gives rise to the primary vascular tissue.

Pycnidia: A flask-shaped or globose fruiting body of fungi lined inside with conidiophores.

* Indicates those words found elsewhere in the glossary.

Pycnidiospores: Asexual spores or conidia produced within a fungal fruiting body called a pycnidium.

Qualitatively: Characterization based on a visual and generally subjective assessment.

Quantitatively: Characterization based on numerical assessment (measurement).

Recalcitrant: A classification of seed based on storability. These seeds cannot be dried down to low moisture contents or stored at subfreezing temperatures. Storability is generally short term (months to years) (compare *Orthodox*).

Relative humidity: A measure of atmospheric humidity, RH refers to the amount of water vapour present in a sample of air, expressed as a percentage of how much water vapour that sample can hold (water holding capacity) at its given temperature. Because water holding capacity changes with temperature, two samples of air at different temperatures may have the same RH but different vapour pressure deficits* (VPDs).

Root apical meristem: A region at the tip of each root in which cell divisions occur to produce new root tissue.

Rootcap: A cone-shaped structure that covers the root apical meristem and protects it as it grows through the soil.

Saprophytic: (of a saprobe) The action of a saprobe, an organism that utilizes dead organic material for food.

Scalper: A multi-screened vibrational seed cleaner used to separate debris from seeds. Screens will vary in size and shape to remove different types of debris.

Scarification: Degradation of the seed coat by mechanical abrasion or chemical treatment to increase the permeability of water and gases or lower the mechanical resistance.

Seed coat: The protective covering of a seed that develops from the integuments* of the ovule* after fertilization.

Select: Seed and vegetative material having a level of gain greater than zero for some trait of interest. Generally, seedlots registered as select are assigned a genetic worth* for a specific trait.

Shoot apical meristem: A region at the tip of each shoot in which cell divisions occur to produce new stem tissue.

Short-day treatment: Refers to the method of application of photoperiod control to induce flowering in short-day plants. In conifers it is used to induce the shoot apical meristem to set (produce) a terminal vegetative bud, thereby causing the cessation of height growth for the duration of the current growing season.

Solutes: A substance dissolved in a solvent forming a solution.

Sowing factor (SF): The average number of seeds per cavity within a container that should be sown to produce a seedling (e.g., 2.2 seeds per cavity).

Specific gravity: The ratio of the density of a substance to the density of water as a standard. The variable is unitless.

Spores: (sing. spore) A general term for a reproductive structure in cryptogams (fungi, algae, mosses, and ferns).

Standard: A reference to seeds that originate from wild stands with no known level of genetic gain (compare *Select*).

Stratification: A technique used to overcome embryo dormancy in seed. Stratification is synonymous with moist-chilling. Seeds are imbibed and then put into cool conditions (2–5°C) for the duration required to overcome dormancy.

Stylar: (of the style) Tissues of the style, the thin part of a pistil between the ovary and the stigma on angiosperms.

Symbionts: Organisms living in symbiosis.

Symbiosis: The association of two different organisms living attached to each other or none within the other.

Symptoms: Something that indicates the presence of something else, usually in reference to a condition or pathogen.

Systemic: Entering a plant via the roots or shoots and passing through the tissues.

Vapour pressure deficit: A measure of atmospheric humidity, VPD refers to the difference between the actual vapour pressure and the maximum vapour pressure possible at the temperature of a sample. In horticulture it can be related to the amount of transpirational draw that a plant experiences.

Vascular cambium: A plant tissue consisting of actively dividing cells that is responsible for increasing the girth of the plant (i.e., it causes secondary growth). The vascular cambium occurs in the stem and root; it divides to produce secondary xylem* and secondary phloem. In mature stems the vascular cambium is extended laterally to form a complete ring.

Vigour: A term used to define the relative robustness of a seedlot. A vigourous seedlot will germinate rapidly over a wide range of conditions. The term is also used on SPAR to describe the germination value (GV).

Water insoluble: Difficult or impossible to dissolve in water.

Water potential: The chemical potential of water measured in bars or megapascals. A diagnostic tool that enables one to assign a value to the water status in plant cells and tissues. The lower the water potential, the greater the ability to absorb moisture.

Wettable powders: consist of finely-ground pesticide active and inert ingredients. Wettable powders must be added to water and kept in suspension through constant agitation. Wettable powders usually contain 50% or more active ingredient.

Xylem: Woody tissues in vascular plants that give support and conduct water and nutrients.

Zygote: A fertilized female gamete. The product of fusion of the nucleus of an ovule with the nucleus of a pollen grain.

* Indicates those words found elsewhere in the glossary.

&

Registration # _____

Agency: _____

Species: _____

Evaluation Type

Pre-collection: _____

Interim Storage: _____

Receipt at TSC: _____ Date Rec'd: _____

TSC Conditioning:

Embryo Comments:

- ❒ 90% Yellow
- ❒ 90% + Pale Yellow
- ❒ 90% + White
- ❒ Other

Storage Tissue Comments:

- ❒ Milky/with shrinkage
- ❒ Firm/with shrinkage
- ❒ Firm/no shrinkage

Seed Wing Comments

- ❒ Cream/Translucent
- ❒ Tan
- ❒ Brown

Easily released from scale ❒ yes ❒ no

Seed Coat Comments

- ❒ Cream
- ❒ Tan Brown
- ❒ Dark Brown

Cones Comments:

- ❒ Green
- ❒ Light Brown
- ❒ Brown
- ❒ Cosed
- ❒ Slight Flex
- ❒ Flexing

Lodgepole Pine: %

Class I _____

Class II _____

Class III _____

Class IV _____

Internal cone colour and condition: _____

Number of filled seed per half cone*: _____

Recommended collection standard: _____

Number of filled seed per cone*: _____

Number of cones sampled: _____

Insect activity/damage*: _____

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Internal						
External						

Fungal Activity: _____

Other: _____

Remarks: _____

_____

_____

_____

Assessed by: _____ Date: _____

Contact: _____ Date: _____ Phone: _____ Fax: _____

* Indicate results/observations by cone, on reverse.

Conifer Seed Sanitation Method Using Hydrogen Peroxide[1]

Non-resin Vesicle Seed: Coastal Douglas-fir and Western Larch

Prior to or following stratification, seeds may be soaked for one to four hours in a 3% hydrogen peroxide (H_2O_2) solution. Mix technical grade 30% hydrogen peroxide with tap water to the appropriate concentration prior to contact with seeds. The seeds should be completely immersed in the newly made 3% solution at a ratio of 3:1 peroxide solution to seed volume. Gentle stirring of the seeds and hydrogen peroxide solution should be performed occasionally. Following the sanitation treatment, seeds should be gently rinsed under running water to remove traces of the hydrogen peroxide solution. Seeds may then be surface-dried, stratified and/or sown.

Resin Vesicle Seed Species: Subalpine Fir

The procedure is essentially the same except the high fungal and bacterial levels on some seedlots may lead to very active foaming of the soaking solution. Also, there is the possibility of damage to resin vesicles resulting in handling problems when the seeds are sown. Therefore, soak times prior to or following stratification should be reduced to a maximum of one hour to reduce these effects. Choose a container with at least twice the volume of seeds plus solution being used. The foaming action of the solution subsides within minutes of the initial contact between the hydrogen peroxide solution and the seeds but a large container will prevent the loss of seeds. In some cases more 3% hydrogen peroxide solution may need to be added to ensure than all seeds are covered in liquid. Rinsing and surface drying should proceed as described above.

Hydrogen Peroxide

Hydrogen peroxide has the desirable quality of rapidly breaking down into water, thereby reducing concerns over environmental contamination. However, a 30% solution is extremely corrosive and must be handled with extreme caution.

Use the concentration chart that follows to obtain the desired volume of 3% dip solution using 30% technical grade hydrogen peroxide and the appropriate volume of water.

Final dip solution 3% (litres)	Hydrogen peroxide 30% (litres)	Water (litres)
10	1.0	9.0
15	1.5	13.5
20	2.0	18.0
25	2.5	22.5
30	3.0	27.0
35	3.5	31.5
40	4.0	36.0
45	4.5	40.5
50	5.0	45.0

To determine a specific amount of stock solution (i.e., 30% hydrogen peroxide) needed to produce a given volume of final dip solution (i.e., 3% hydrogen peroxide) use the following formula:

$$\text{Volume of stock solution} = \frac{\text{volume total of dip solution} \times \text{dip concentration}}{\text{stock concentration}}$$

Total volume of dip solution = volume of stock solution + water

Example: How much 30% H_2O_2 (stock) is required to produce 22 litres of 3% H_2O_2 as a final dip solution.

$$\text{Volume of stock solution} = \frac{22 \text{ litres} \times 3}{30} = 2.2 \text{ litres}$$

...add 2.2 litres of 30% H_2O to 19.8 (22–2.2) litres of water.

Precautions – Hydrogen Peroxide

Avoid contact and inhalation. Wear chemical goggles. Avoid the use of contact lenses. Wear a long sleeved shirt, trousers, rubber boots, rubber gloves, and a rubber apron. Hydrogen peroxide is a *corrisive oxidizer*. Keep away from chromium, manganese, silver, platinum, and palladium. Keep away from organics, sodium borate, urea, sodium carbonate, sodium fluoride, and sodium pyrophosate. Provide adequate ventilation and use a multi-gas detector to monitor the air above the rinse tank. The Workers' Compensation Board of British Columbia *Industrial Health & Safety Regulations* permissible concentration for an eight-hour exposure is 1 ppm.

For a complete list of safety precautions and disposal recommendations refer to the manufacturer's material safety data sheet. Observe all federal, provincial, and local waste disposal laws and regulations.

[1] Modified from Neumann (1997) and Peterson (1991).

Equipment Cleaning Methods[2]

Sanitation Method Using Ivory® Dishwashing Liquid and Hot Water

Ideally, cleaning should occur weekly, especially during times of high use. However, as this may not always be possible, cleaning every two weeks would be beneficial as this will help to reduce the build-up of *Fusarium* spp. inoculum levels. Tank cleaning should begin just prior to the beginning of the seed preparation season and continue until the end of the season.

A 5% solution of Ivory®, dishwashing soap and very hot water (200 ml Ivory, and 3800 ml hot [50°C] water) should be made up just prior to use. Seed processing containers or tanks should be scrubbed with special care being given to the corners and bottom surface since that is where most of the contamination is present. Container/tanks should be rinsed thoroughly with cold water. To ensure complete removal of soapsuds, the container/tank should be filled partially with water to dilute the soap and to speed its removal.

*Precautions – **Dishwashing Soap***

Care should be taken to avoid eye contact with the soap product. Splash-resistant safety goggles/glasses should be worn. Spillage of soap solution onto floors may cause them to be slippery temporarily. Thorough rinsing of tanks will ensure that disposal of soap occurs with a minimum of foam.

Screen Cleaning Method Using Bleach, Buffer, and Water

A 0.5% bleach and buffer solution should be made up with cold water of a sufficient volume to soak and completely cover seed-soaking screens. The solution should be made of equal parts of buffer to bleach (12%) and made up with enough cold water to produce a 0.5% solution of the required volume. A typical solution would contain 160 ml of 12% industrial sodium hypochlorite, 160 ml of buffer (75% phosphoric acid, 50% sodium hydroxide); and 3840 ml of cold water. Screens should be soaked for 3 to 4 hours and then rinsed thoroughly for at least 30 minutes in running tap water. For maximum efficiency, screens could be cleaned every two weeks but monthly will reduce inoculum loads as well.

Bleach

A 0.5% bleach solution is a more effective sanitising agent when buffered to a pH of 7.0. Either household bleach (6% available chlorine) or industrial bleach (12% available chlorine) can be used. The pH of the bleach solution can be assessed with pH paper or a pH meter. The pH should be close to 7.0.

If the pH is above 7.0, slowly add buffer.

If the pH is below 7.0, slowly add bleach.

Use the concentration charts below to obtain a 0.5% dip solution at the volume desired by adding equal parts buffer to bleach (12%) with the appropriate volume of water.

Final dip solution (litres)	Bleach 12% (litres)	Buffer (litres)	Water (litres)
10	0.4	0.4	9.2
15	0.6	0.6	13.8
20	0.8	0.8	18.4
25	1.0	1.0	23.0
30	1.2	1.2	27.6
35	1.5	1.5	32.0
40	1.7	1.7	36.6
45	1.9	1.9	41.2
50	2.1	2.1	45.8

Use the following chart to obtain a 0.5% dip solution at the volume desired by adding one part buffer to two parts bleach (6%) with the appropriate volume of water.

Final dip solution (litres)	Bleach 6% (litres)	Buffer (litres)	Water (litres)
10	0.8	0.4	8.8
15	1.3	0.6	13.1
20	1.7	0.8	17.5
25	2.1	1.0	21.9
30	2.5	1.2	26.3
35	2.9	1.5	30.6
40	3.3	1.7	35.0
45	3.8	1.9	39.4
50	4.2	2.1	43.8

*Precautions – **Bleach***

Inhalation of fumes or mists causes respiratory tract and mucous membrane irritation. Liquid and mists will irritate the skin or damage the eyes. Wear chemical goggles. Avoid the use of contact lenses. Wear long-sleeved shirt, trousers, rubber boots, rubber gloves, and rubber apron.

The chlorine concentration in the atmosphere above the rinse tank can be monitored using a multi-gas detector. The Workers' Compensation Board of British Columbia *Industrial Health & Safety Regulations* permissible concentration for an eight-hour exposure is 1 ppm.

For a complete list of safety precautions and disposal recommendations refer to the manufacturer's material safety data sheet. Observe all federal, provincial, and local waste disposal laws and regulations.

2 Modified from Neumann (1997) and Peterson (1991).

Conifer Seed Sanitation Method Using Hydrogen Peroxide[1]

Non-resin Vesicle Seed: Coastal Douglas-fir and Western Larch

Prior to or following stratification, seeds may be soaked for **one to four hours** in a 3% hydrogen peroxide (H_2O_2) solution. Mix technical grade 30% hydrogen peroxide with tap water to the appropriate concentration prior to contact with seeds. The seeds should be completely immersed in the newly made 3% solution at a ratio of 3:1 peroxide solution to seed volume. Gentle stirring of the seeds and hydrogen peroxide solution should be performed occasionally. Following the sanitation treatment, seeds should be gently rinsed under running water to remove traces of the hydrogen peroxide solution. Seeds may then be surface-dried, stratified and/or sown.

Resin Vesicle Seed Species: Subalpine Fir

The procedure is essentially the same except the high fungal and bacterial levels on some seedlots may lead to very active foaming of the soaking solution. Also, there is the possibility of damage to resin vesicles resulting in handling problems when the seeds are sown. Therefore, soak times prior to or following stratification should be reduced to a maximum of one hour to reduce these effects. Choose a container with at least twice the volume of seeds plus solution being used. The foaming action of the solution subsides within minutes of the initial contact between the hydrogen peroxide solution and the seeds but a large container will prevent the loss of seeds. In some cases more 3% hydrogen peroxide solution may need to be added to ensure than all seeds are covered in liquid. Rinsing and surface drying should proceed as described above.

Hydrogen Peroxide

Hydrogen peroxide has the desirable quality of rapidly breaking down into water, thereby reducing concerns over environmental contamination. However, a 30% solution is extremely corrosive and must be handled with extreme caution.

Use the concentration chart that follows to obtain the desired volume of 3% dip solution using 30% technical grade hydrogen peroxide and the appropriate volume of water.

Final dip solution 3% (litres)	Hydrogen peroxide 30% (litres)	Water (litres)
10	1.0	9.0
15	1.5	13.5
20	2.0	18.0
25	2.5	22.5
30	3.0	27.0
35	3.5	31.5
40	4.0	36.0
45	4.5	40.5
50	5.0	45.0

To determine a specific amount of stock solution (i.e., 30% hydrogen peroxide) needed to produce a given volume of final dip solution (i.e., 3% hydrogen peroxide) use the following formula:

$$\text{Volume of stock solution} = \frac{\text{volume total of dip solution} \times \text{dip concentration}}{\text{stock concentration}}$$

Total volume of dip solution = volume of stock solution + water

Example: How much 30% H_2O_2 (stock) is required to produce 22 litres of 3% H_2O_2 as a final dip solution.

$$\text{Volume of stock solution} = \frac{22 \text{ litres} \times 3}{30} = 2.2 \text{ litres}$$

...add 2.2 litres of 30% H_2O to 19.8 (22–2.2) litres of water.

*Precautions – **Hydrogen Peroxide***

Avoid contact and inhalation. Wear chemical goggles. Avoid the use of contact lenses. Wear a long sleeved shirt, trousers, rubber boots, rubber gloves, and a rubber apron. Hydrogen peroxide is a *corrisive oxidizer.* Keep away from chromium, manganese, silver, platinum, and palladium. Keep away from organics, sodium borate, urea, sodium carbonate, sodium fluoride, and sodium pyrophosate. Provide adequate ventilation and use a multi-gas detector to monitor the air above the rinse tank. The Workers' Compensation Board of British Columbia *Industrial Health & Safety Regulations* permissible concentration for an eight-hour exposure is 1 ppm.

For a complete list of safety precautions and disposal recommendations refer to the manufacturer's material safety data sheet. Observe all federal, provincial, and local waste disposal laws and regulations.

[1] Modified from Neumann (1997) and Peterson (1991).

Equipment Cleaning Methods[2]

Sanitation Method Using Ivory® Dishwashing Liquid and Hot Water

Ideally, cleaning should occur weekly, especially during times of high use. However, as this may not always be possible, cleaning every two weeks would be beneficial as this will help to reduce the build-up of *Fusarium* spp. inoculum levels. Tank cleaning should begin just prior to the beginning of the seed preparation season and continue until the end of the season.

A 5% solution of Ivory®, dishwashing soap and very hot water (200 ml Ivory, and 3800 ml hot [50°C] water) should be made up just prior to use. Seed processing containers or tanks should be scrubbed with special care being given to the corners and bottom surface since that is where most of the contamination is present. Container/tanks should be rinsed thoroughly with cold water. To ensure complete removal of soapsuds, the container/tank should be filled partially with water to dilute the soap and to speed its removal.

Precautions – ***Dishwashing Soap***

Care should be taken to avoid eye contact with the soap product. Splash-resistant safety goggles/glasses should be worn. Spillage of soap solution onto floors may cause them to be slippery temporarily. Thorough rinsing of tanks will ensure that disposal of soap occurs with a minimum of foam.

Screen Cleaning Method Using Bleach, Buffer, and Water

A 0.5% bleach and buffer solution should be made up with cold water of a sufficient volume to soak and completely cover seed-soaking screens. The solution should be made of equal parts of buffer to bleach (12%) and made up with enough cold water to produce a 0.5% solution of the required volume. A typical solution would contain 160 ml of 12% industrial sodium hypochlorite, 160 ml of buffer (75% phosphoric acid, 50% sodium hydroxide); and 3840 ml of cold water. Screens should be soaked for 3 to 4 hours and then rinsed thoroughly for at least 30 minutes in running tap water. For maximum efficiency, screens could be cleaned every two weeks but monthly will reduce inoculum loads as well.

Bleach

A 0.5% bleach solution is a more effective sanitising agent when buffered to a pH of 7.0. Either household bleach (6% available chlorine) or industrial bleach (12% available chlorine) can be used. The pH of the bleach solution can be assessed with pH paper or a pH meter. The pH should be close to 7.0.

If the pH is above 7.0, slowly add buffer.

If the pH is below 7.0, slowly add bleach.

Use the concentration charts below to obtain a 0.5% dip solution at the volume desired by adding equal parts buffer to bleach (12%) with the appropriate volume of water.

Final dip solution (litres)	Bleach 12% (litres)	Buffer (litres)	Water (litres)
10	0.4	0.4	9.2
15	0.6	0.6	13.8
20	0.8	0.8	18.4
25	1.0	1.0	23.0
30	1.2	1.2	27.6
35	1.5	1.5	32.0
40	1.7	1.7	36.6
45	1.9	1.9	41.2
50	2.1	2.1	45.8

Use the following chart to obtain a 0.5% dip solution at the volume desired by adding one part buffer to two parts bleach (6%) with the appropriate volume of water.

Final dip solution (litres)	Bleach 6% (litres)	Buffer (litres)	Water (litres)
10	0.8	0.4	8.8
15	1.3	0.6	13.1
20	1.7	0.8	17.5
25	2.1	1.0	21.9
30	2.5	1.2	26.3
35	2.9	1.5	30.6
40	3.3	1.7	35.0
45	3.8	1.9	39.4
50	4.2	2.1	43.8

Precautions – ***Bleach***

Inhalation of fumes or mists causes respiratory tract and mucous membrane irritation. Liquid and mists will irritate the skin or damage the eyes. Wear chemical goggles. Avoid the use of contact lenses. Wear long-sleeved shirt, trousers, rubber boots, rubber gloves, and rubber apron.

The chlorine concentration in the atmosphere above the rinse tank can be monitored using a multi-gas detector. The Workers' Compensation Board of British Columbia *Industrial Health & Safety Regulations* permissible concentration for an eight-hour exposure is 1 ppm.

For a complete list of safety precautions and disposal recommendations refer to the manufacturer's material safety data sheet. Observe all federal, provincial, and local waste disposal laws and regulations.

2 Modified from Neumann (1997) and Peterson (1981).

Seed Handling and Safety

Other Seed Contaminants

Depending on the source and species, seed screening assays may find a variety of other fungi and bacteria associated with a particular seedlot. These organisms are primarily moulds that are facultative saprophytes and generally have little host specificity. Most of the species belong to the genera *Aspergillus, Chaetomium, Mucor, Penicilium* and *Rhizopus*. In particular, *Penicillium* and *Aspergillus* are occasionally connected with health effects when found in high concentrations in homes, office buildings and schools. *Aspergillus* produces aflatoxins that are toxic to humans. *Penicillium* may also cause health consequences, especially in individuals who have allergic reactions to the drug penicillin. Another candidate is *Stachybotrys*. In recent years, it has been a source of concern in chronically wet buildings that have considerable amounts of cellulose-based materials.

Some of these fungi may cause allergies. An allergic reaction occurs when a substance provokes formation of antibodies in a susceptible person. These substances that cause an allergic reaction are termed *antigens or allergens*. Rashes, hay fever, asthma (tightness in the chest, difficulty in breathing), and runny noses are common allergic reactions.

Occupational Hazardous Fungi

Aspergillus

A genus of fungi with over 100 species of which approximately 15 are commonly encountered in dwellings. Most naturally occurring *Aspergilli* are toxigenic or allergenic. Among several toxigenic species of this genus, the most important are, *A. parasiticus, A. flavus, A. versicolor* and *A. fumigatus*. Aflatoxins are among the most extensively studied mycotoxin.

Penicillium

Some *Penicillium* species are fairly common indoor fungi, even in clean environments. These particular species of fungi can proliferate in sub-basement offices and rooms, in libraries, auditorium, storage room of paper materials and also in ventilation systems. Some *Penicillium* species can produce small, non-descript conidia and complex mixtures of metabolites that are more or less toxic. Like all other moulds, spores have the highest concentrations of mycotoxin, although the vegetative portion of the mycelium can also contain the material.

Stachybotrys

It is a genus of moulds (Hyphomycetes) characterized by having a high moisture requirement so it grows in chronically wet areas. This mould has a very low nitrogen requirement, and can grow on water-saturated cellulosic materials such as paper, wallpaper, ceiling tiles, carpets, insulation material, wood-derived building materials and even general debris.

Control Strategies

Most of these associated organisms are either benign or in insufficient numbers to affect people working with seed sources. Yet it is prudent to implement some simple procedures to ensure the safety of any individuals working in the seed processing area:

- Designate an area in your production facilities as a seed processing lab.
- Use water/chemical resistant materials on the benching and floor.
- Reduce relative humidity. Use a chemical or mechanical dehumidifier.
- If you find mould or mildew in your seed processing area, try to find and eliminate sources of moisture.
- Clean mould and mildew growth from bench surfaces, walls, floors with water mixed with chlorine bleach, diluted to 0.5% solution bleach (see table on previous page). Commercial products can also remove mildew and mould. Follow product instructions carefully. This should be done on a regular basis. Very mouldy items should be replaced.
- Vent to the outside.
- Change heating and cooling system filters monthly.
- Vacuum air return covers or screens regularly.
- Check air conditioners for mould before each cooling season and have coils cleaned as needed.
- Have heating/cooling system ductwork checked for loose insulation, leaks, or signs of condensation where the system enters the building. Insulate ducts on the outside.
- Consider installing air cleaners and filters. Electronic and hePA (high efficiency particulate absolute) cleaners and filters are best at taking mould, mildew, and dust out of the air.
- Use simple personal protection such as latex gloves, eye protectors, lab coat and if necessary, wear an appropriate respiratory filter.

Germination Stages 1 & 2

	Stage 1	Stage 2
Temperature at seed level	15–25°C – optimum >20°C – no day/night differential required	15–25°C – optimum >20°C – day/night differential photoperiod dependent
Light intensity	≤50% full sun	≥50% full sun – depends on time of year – depends on latitute – depends on GH cover
Photoperiod	20+ hour extended day	20+ hour extended day
Moisture	– even, moist to wet – media not saturated – maintain media aeration porosity	– moist
Fertilization	– 10–30 PPM NO_3 based – starter/grower formulation – acts as seed primer when watering	– 20–50 PPM NO_3 based – starter/grower formulation – radicle may be taking up
Grit cover	– maintains constant °C and RH – helps orient emerging radicle – reduces moss and algae growth – 3 mm forestry sand	– maintains constant °C and RH – helps orient emerging radicle – reduces moss and algae growth – 3 mm forestry sand
RH	95%+	85–95% – light dependent
pH	4.5–6.0 4.5–6.0	
Air circulation	– minimal	– increase
CO_2 injection	– no	– minimal if desired – wait until green is evident – include air circulation – light dependent to 800 PPM
Monitor EC	<0.5 mmhos/cm^3	≤0.5 mmhos/cm^3

Germination Stages 1 & 2

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RH	95%+	85–95% - light dependent
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Air circulation	- minimal	- increase
CO_2 injection	- no	- minimal if desired - wait until green is evident - include air circulation - light dependent to 800 PPM
Monitor EC	<0.5 mmhos/cm^3	≤0.5 mmhos/cm^3

Distance

1 millimetre [mm]		0.0394 in
1 centimetre [cm]	10 mm	0.3937 in
1 metre [m]	100 cm	1.0936 yd
1 kilometre [km]	1000 m	0.6214 mile
1 inch [in]		2.54 cm
1 foot [ft]	12 in	0.3048 m
1 yard [yd]	3 ft	0.9144 m
1 mile	1760 yd	1.6093 km
1 int nautical mile	2025.4 yd	1.852 km

Area

1 sq cm [cm^2]	100 mm^2	0.1550 in^2
1 sq m [m^2]	10 000 cm^2	1.1960 yd^2
1 hectare [ha]	10 000 m^2	2.4711 acres
1 sq km [km^2]	100 ha	0.3861 $mile^2$
1 sq inch [in^2]		6.4516 cm^2
1 sq foot [ft^2]	144 in^2	0.0929 m^2
1 sq yd [yd^2]	9 ft^2	0.8361 m^2
1 acre	4840 yd^2	4046.9 m^2
1 sq mile [$mile^2$]	640 acres	2.59 km^2

Volume/Capacity

1 cu cm [cm^3]		0.0610 in^3
1 cu decimetre [dm^3]	1000 cm^3	0.0353 ft^3
1 cu metre[m^3]	1000 dm^3	1.3080 yd^3
1 liter [l]	1 dm^3	1.76 pt
1 hectolitre [hl]	100 l	21.997 gal
1 cu inch [in^3]		16.387 cm^3
1 cu foot [ft^3]	1728 in^3	0.0283 m^3
1 fluid ounce [fl oz]		28.413 ml
1 pint [pt]	20 fl oz	0.5683 l
1 gallon [gal]	8 pt	4.5461 l
1 fluid ounce	1.0408 UK fl oz	29.574 ml
1 pint (16 fl oz)	0.8327 UK pt	0.4731 l
1 gallon	0.8327 UK gal	3.7854 l
1 bushel		35.239 l

Mass

1 milligram [mg]		0.0154 grain
1 gram [g]	1000 mg	0.0353 oz
1 kilogram [kg]	1000 g	2.2046 lb
1 tonne [t]	1000 kg	0.9842 ton
1 kg/hectare		0.892 lbs/acre
1 ounce [oz]	437.5 grain	28.35 g
1 pound [lb]	16 oz	0.4536 kg
1 stone	14 lb	6.3503 kg
1 hundredweight [cwt]	112 lb	50.802 kg
1 ton	20 cwt	1.016 t
1 pound/acre		1.12 kg/hectare

Temperature

Degrees Celsius	Degrees Fahrenheit	(degrees C × 9/5) + 32
Degrees Celsius	Kelvin	(degrees C + 273.16)
Degrees Fahrenheit	Degrees Celsius	(degrees F - 32) × 5/9
Degrees Fahrenheit	Degrees Rankin	(degrees F + 459.69)